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PHYSIOLOGIC SPECIALIZATION IN THE LEAF RUST OF WHEAT, PUCCINIA TRITICINA ERIKSS.[1]

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WITH PLATES VI TO IX

INTRODUCTION

A study of the leaf rust of wheat, caused by *Puccinia triticina* Erikss., was begun in 1918 in cooperation with the Office of Cereal Investigations, United States Department of Agriculture. The results obtained in a study of the life history of this rust have already been reported (13). As another phase of the investigation, a study has been made to determine the susceptibility of wheat varieties to leaf rust with the object of obtaining resistant varieties. In order to determine the value of the resistant varieties of wheat, it became necessary to make an intensive investigation to determine whether the rust, as it is found in this country, is homogeneous or whether it consists of a number of forms differing in their pathogenicity upon different varieties. The results obtained by Eriksson and Henning (7), Eriksson (5, 6), Hitchcock and Carleton (11), Carleton (2), Freeman and Johnson (8), and Jackson and Mains (13) indicate that the leaf rust of wheat is very closely restricted as to hosts. Wheat has been found to be the only favorable grass host for the uredinial and telial stages, and a few species of *Thalictrum* are the hosts of the aecial stage.

The question as to whether there exist in the leaf rust of wheat specialized forms distinguished by their parasitism, similar to those which have

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been found by Stakman and coworkers (14, 23, 24) and Melchers and Parker (18) in connection with their studies on *Puccinia graminis tritici* Erikss. and Henn., has been unanswered up to the present. The investigations of the stem rust of wheat show that such strains are of great importance in breeding for stem-rust resistance, and that a knowledge of them is necessary before it is possible to draw conclusions as to the value of resistant varieties. This paper deals with the results obtained in a study of this problem as it relates to leaf rust of wheat and which have been reported in part before the American Phytopathological Society (16, 17).

METHODS

Collection of inoculum. When the leaf rust was collected locally, the method usually employed was to bring in rusted leaves of wheat and sow the spores at once on healthy seedlings. When inoculum was obtained from other localities, rusted leaves were placed in heavy manila envelopes and mailed to the laboratory at LaFayette, Ind. It was not found necessary to wrap the leaves in oiled paper to prevent drying; in fact, the germinability of the spores is better if oiled or moistened paper is not used for wrapping. It was found, also, that inoculum mailed without precautions against drying would retain its viability for a number of months (15) if placed in an ice-box. In this way it was possible to collect the rust at a number of places before harvest, store it during the hot summer months when it is difficult to culture the rust in the greenhouse, and then start cultures in the autumn.

Inoculation. A susceptible variety of wheat was used on which to maintain the rust cultures. At first Dawson Goldenchaff was used; later, Michigan Amber and Big Harvest Fultz were employed. Usually about ten plants were grown in a four-inch pot. Previous to inoculation all plants were kept in a small greenhouse, in which no rust culture was allowed, and were carried from there to another greenhouse where they were inoculated and studied. One of the difficulties encountered early in the course of the work was the development of powdery mildew of wheat, *Erysiphe graminis tritici* Marchal, which often made culture study difficult. If plants bearing stock cultures of the rust became infected with mildew, the mildew was sown with the rust and interfered with the study of the rust susceptibility of the wheat varieties. This difficulty was overcome in two ways. The hot-water heating pipes in the small greenhouse where uninoculated plants were grown were painted with a mixture of lime and sulphur. The sulphur fumes thus generated proved very effective in preventing the development of mildew. A study of wheat varieties also resulted in revealing several which were highly resistant to mildew. Among these was a strain of Michigan Amber which was also very susceptible to rust. The complications

caused by the mildew may be avoided by using this strain as the variety for stock cultures.

The wheat seedlings were inoculated when in about the second-leaf to fourth-leaf stage. This was done by first drawing the leaves between the moistened fingers, thus removing the waxy covering and leaving a film of water on the leaves. Spores were scraped from rusted wheat and spread on these wet leaves by means of a scalpel. When only one or two plants were inoculated, they were placed under a bell jar or plant cover. When a larger number were inoculated, they were placed in galvanized iron tanks. A high humidity (practically 100 per cent) was maintained by placing a layer of wet sphagnum in the bottom. The tanks were covered by a lid in which a pane of glass was inset to allow entrance of light and prevent etiolation of the wheat seedlings. The plants were kept in these inoculation chambers for 48 hours, being atomized with water morning and evening to help maintain the humidity and keep water on the wheat leaves. This is necessary because a high humidity and actual contact with condensed water are two of the conditions necessary for spore germination. Temperature also is an important factor. Temperatures between 16 and 20° C. are most favorable for the spore germination of leaf rust of wheat, while little germination takes place at 5° C. or below, and 30° C. or above. When the temperature of the greenhouse was too high for the best spore germination, as sometimes occurs in late spring, summer, or early fall, inoculation chambers were cooled to optimum conditions by keeping them sprayed with water by means of a system of spray nozzles attached to the greenhouse water supply. When the inoculated plants were removed, it was found advisable not to place them in full sunlight, as they were likely to be scalded. Consequently, they usually were taken from the inoculation chambers in the late afternoon.

As several different cultures of leaf rust were being studied at the same time, for possible differences in parasitism, it was necessary to take precautions to prevent contamination of one by the other. Consequently after sowing one culture and before sowing the next, all instruments and vessels were sterilized in alcohol or in cleaning fluid, and the hands thoroughly washed. The cultures were placed in separate incubation chambers and later removed to separate plant covers or tent-like chambers. The sides and ends of the chambers are of cypress and the roof of two panes of glass which slide up and down in grooves, thus permitting easy access to the culture with minimum exposure to wind-blown spores.

Uredinia of leaf rust usually appear in 6 to 10 days, depending upon conditions of light and temperature. After the first uredinia have appeared, others are often developed later in a circle around them. The culture continues to sporulate as long as the infected leaf remains alive,

which varies with the amount of rust, the available food supply of the leaf, and especially the environmental conditions affecting the latter. As conditions are not favorable for spore germination in the plant cover, humidity especially being low, reinfection does not occur, and it is necessary to resow the rust. This is usually done on fresh pot cultures of wheat seedlings. Such transfers are necessary about every 4 to 6 weeks.

From the cultures of rust maintained in the plant covers, sowings were made on pots of seedlings of various varieties of wheat. Inoculation was carried out as described above, with the exception that, when the sets of varieties were too large to place under plant cover after removal from the incubation chambers, they were placed on the greenhouse bench. Further inoculations with the cultures were always from the susceptible variety protected from contamination within the plant cover.

Pure lines of leaf rust. In the course of the investigation, several of the cultures gave evidence of being composed of more than one physiologic form of the rust. Consequently it became necessary to devise a method of obtaining pure cultures or pure lines of the rust, in order to be sure that the results obtained were really characteristic of the forms and not due to a mixture of two or more forms. The method finally used was to obtain cultures of the rust from single urediniospores.

Wheat was sown in four-inch flower pots and immediately covered with a lantern globe, the top of which was covered with a thin layer of cotton held in place by a piece of cheesecloth tied over the top. The lantern globe fitted down tightly into the soil of the pot; consequently there was practically no chance of the wheat seedlings which came up within becoming contaminated from outside spores. When these plants were large enough to inoculate, a rust-infected leaf was gently tapped over a Petri dish, so that ripe urediniospores were scattered over the bottom of the dish. This dish was then placed under the higher powers of a binocular microscope and a single isolated urediniospore located. This was picked up by means of a glass rod drawn to a fine point; the point was wet in water, and the spore usually adhered readily. This spore was then transferred to a leaf of one of the plants inside the lantern globe. Each of the plants in the globe was inoculated with a single spore. The inoculated plants were then carefully watched, and, with the appearance of the first uredinium, all the other plants under the globe were removed. When this uredinium reached maturity, urediniospores were sown by means of a scalpel upon other plants grown within a lantern globe, thus maintaining the culture. Several such cultures were started from single spores. The inoculum produced under such lantern globes, although limited, is sufficient to inoculate from six to ten pots of wheat seedlings. When larger numbers were to be inoculated,

the culture was first multiplied on the susceptible variety under a plant cover and from this the series was inoculated. However, the culture under the lantern globe was also continued, and, if further sets of varieties were to be inoculated, the rust was again multiplied and sown from this, thus insuring the purity of the culture.

In making single-spore cultures, a difficulty was encountered in the late fall and winter. During this period of low light intensity, the percentage of germination of urediniospores is usually rather low, oftentimes being only 10 per cent. It readily can be seen that, where only ten spores are sown, there is considerable chance that germinable spores will not be selected and thus no culture obtained. This frequently resulted, and the task of obtaining single-spore cultures proved a very laborious one, for it was necessary to inoculate with a large number of single spores. It was found, however, that if the Petri dish containing spores was covered and set aside in the laboratory for two or three days, the percentage of germinable spores increased greatly and at least one of the ten inoculated plants under the lantern globe usually became infected.

Another difficulty encountered is that of maintaining single-spore cultures during the late spring and summer months, when the temperature of the greenhouse naturally is high and especially so under the lantern globes. Such conditions do not favor the growth of wheat. Wheat under the lantern globes becomes infected also by a number of fungi, especially *Helminthosporium* spp., and as a result the plants are killed and many cultures lost. Also, during this period, the leaf rust of wheat is very prevalent in the field. In most seasons nearly all the varieties of wheat generally grown in the eastern United States are more or less heavily infected with leaf rust. Consequently during this period urediniospores are very prevalent in the air. Stakman *et al.* (22) report finding them as high as 10,000 feet. With the greenhouse ventilators necessarily open at this time of year, when single-spore cultures are carried on wheat, except under very confined conditions, they become contaminated by these wind-carried spores. It therefore has been very difficult to carry the cultures from one year to another. Since, however, it has been found that the urediniospores will retain their viability to some extent for several months, it is possible to collect the urediniospores of each culture, store them through the summer in vials in an icebox and start the culture anew in the autumn.[3] Although some cultures are lost, this method has made it possible to carry over from year to year a number of the cultures with a reasonable certainty that they were pure.

[3] The effect of drying on spore germination and the method of storing inoculum have been worked out in connection with studies upon spore germination being made by Mr. Leroy Compton under the direction of the writers.

The data which had been accumulated from a study of the susceptibility of wheat varieties to rust as collected in the field made it possible to select a set of varieties which would be useful to differentiate any strains which might be present. It was possible, also, when making single spore cultures, to select those which differed most widely in their effects on such varieties, thus obtaining a series of cultures which in general were found to be different specialized forms of the rust.

RESULTS

Types of reaction shown by wheat to leaf rust. As but few data were available on the susceptibility of wheat varieties to the leaf rust of wheat, it was first necessary to determine which of the many varieties and strains were resistant to this rust. Therefore approximately 300 varieties and strains of wheat were obtained in 1918 and studied for susceptibility to 10 cultures of leaf rust obtained from different parts of the country. Of these varieties, the most promising were selected for further study. During 1919, 1920, and 1921, data concerning the susceptibility of wheat varieties to leaf rust were obtained from various wheat nurseries, the most extensive being that of the Office of Cereal Investigations at Arlington Experimental Farm, Washington, D. C. Those varieties showing the least rust were studied for susceptibility to leaf rust in the greenhouse during the winter months. Cultures of rust from various parts of the country were obtained for this purpose. It is not proposed in this paper to give an account of the results obtained with all the wheats studied. However, 550 varieties or selections covering a wide range of wheats, including most of the principal American varieties of *Triticum aestivum* L., *T. compactum* Hort., *T. durum* Desf., *T. dicoccum* Schrb., *T. spelta* L., *T. turgidum* L., *T. polonicum* L., and *T. monococcum* L. were inoculated with leaf rust. One hundred and eighteen cultures of leaf rust were used. Naturally all of these cultures were not sown upon all of the varieties and selections. As the study progressed, the number of varieties of wheat was greatly reduced, only those showing more or less marked resistance to one or more of the cultures of the rust being retained.

One of the interesting facts developed in this study was the reaction of a number of varieties showing more or less resistance in the field. Thus, Kanred, which has been noted by Melchers and Parker (18) and by the writers (16) to be usually highly resistant under field conditions, has not shown in general a corresponding high resistance when inoculated in the seedling stage in the greenhouse. Thus, in 1920, Kanred showed only a trace to 10 per cent of rust in nurseries at Madison, Wis., and Washington, D. C. Again in 1921, Kanred showed only a trace to 35 per cent in nur-

series at Murfreesboro, Tenn., Brookings, S. D., Washington, D. C., La-Fayette, Ind., and Madison, Wis. Kanred was well exposed to infection, since a number of varieties nearby showed 75 to 100 per cent infection. Seedlings of Kanred, when inoculated with collections of rusts from each of these nurseries, were more or less susceptible, varying in reaction from 2 to 4 in the seedling stage in the greenhouse. Other varieties, including some of the durums and emmers, while having some resistance in the seedling condition in the greenhouse, were generally almost immune in the field. This was true especially with certain of the cultures. In general, the number of varieties found to be highly resistant in these greenhouse studies was relatively small.

In the course of this study several different kinds of host reaction were found in the varieties studied in the seedling stage in the greenhouse. These are grouped into five classes with five corresponding types of infection, designated by the symbols 0 to 4, as follows:

Classes of host reaction (See Plate VI)	Types of rust infection
0 Highly resistant	No uredinia formed; small flecks, chlorotic or necrotic areas more or less prevalent.
1 Very resistant	Uredinia few, small, always in small necrotic spots. Also more or less necrotic areas produced without development of uredinia.
2 Moderately resistant	Uredinia fairly abundant, of moderate size, always in necrotic or very chlorotic spots. Necrotic spots seldom without uredinia.
3 Moderately susceptible	Uredinia fairly abundant, of moderate size. No necrosis produced, but sometimes slight chlorosis immediately surrounding the uredinia.
4 Very susceptible	Uredinia abundant, large. No necrosis or chlorosis immediately surrounding the uredinia. Infected areas sometimes occurring as green islands surrounded in each case by a chlorotic ring.

Reaction of wheat varieties to physiologic forms. The first indication of the existence of physiologic forms of leaf rust of wheat was discovered through the inoculation of Malakoff, C. I. 4898, with a culture of rust from Murfreesboro, Tenn. Malakoff had hitherto shown a high degree of resistance to fifteen other cultures of leaf rust with which it had been inoculated. To the culture of rust from Murfreesboro, however, it was highly

susceptible. As other cultures were obtained, some were found to which this variety was resistant, while to others it was highly susceptible. In a similar way, the following were the principal varieties found to show differences in reaction to different cultures of the rust: Turkey (Malakoff), C. I. 4898[4]; Turkey 47 (received from Kansas labeled as Turkey × Bearded 47); Norka, C. I. 4377; Mediterranean, C. I. 3332; Mediterranean, C. I. 5776; Mediterranean (Imperial Amber), C. I. 4860; Hussar, C. I. 4843; Democrat, C. I. 3384; Beloglina, C. I. 1544; Beloglina, C. I. 2239; Pacific Bluestem, C. I. 4821; Banat, C. I. 4879; Hungarian, C. I. 4830; four unnamed spring wheats, C. I. Nos. 3747, 3756, 3778 and 3779; Webster, C. I. 3780; Peliss, C. I. 1584; Acme, C. I. 5284; Arnautka, C. I. 1493; Arnautka, Minn. 337; Monad, C. I. 3330; Mindum, C. I. 5296; Khapli emmer, C. I. 4013; Vernal emmer, S. D. 293; Bearded Winter spelt, C. I. 1724; White Winter spelt, C. I. 2968; Rivet, C. I. 4201; Titanic, C. I. 5535; Polish, C. I. 5524; Einkorn, C. I. 2433; and Double Einkorn "Double emmer" from Ottawa.

The identity of these varieties has been checked by Dr. C. E. Leighty, of the Office of Cereal Investigations. Descriptions of most of them are given by Clark, Martin and Ball (3) in their classification of American wheat varieties. As the unnamed or recently named varieties are not described in that publication, the following description of each has been kindly furnished by Dr. Leighty. They are introductions received from St. Petersburg, Russia, in 1912.

C. I. No. 3747. Plant spring habit; stem white; spike awned, fusiform, lax, speltoid; glumes glabrous, brownish, long, midwide, distinctly keeled throughout entire length; shoulders narrow, oblique; beaks 1 to 18 mm. long; awns 3 to 7 mm. long; kernels red, long, semihard.

C. I. No. 3756. Plant spring habit, midseason, midtall; stem white; spike awned, fusiform, lax, speltoid, inclined; glumes glabrous, white, midlong, wide, distinctly keeled throughout entire length; shoulders wide, elevated; beaks 8 to 20 mm. long; awns 3 to 8 mm. long; kernels red, long, hard.

C. I. No. 3778. Plant spring habit; stem white; spike awned, fusiform, lax, speltoid; glumes glabrous, brown, midlong, midwide; shoulders midwide, square; beaks 1 to 5 mm. long; awns 3 to 8 mm. long; kernels red, midlong, hard.

C. I. No. 3779. Plant spring habit; stem white; spike awned, fusiform, lax, speltoid; glumes glabrous, brown, long, midwide; shoulders midwide,

[4] Numbers preceded by "C. I." are the accession numbers of the Office of Cereal Investigations, U. S. Department of Agriculture. Those labeled "Minn." are of the Minnesota Agricultural Experiment Station. Those designated "S. D." are of the South Dakota Agricultural Experiment Station.

square; beaks 3 to 20 mm. long; awns 3 to 8 mm. long; kernels red, midlong to long, hard.

Webster, C. I. No. 3780. Plant spring habit, midseason, midtall; stem white, weak; spike awned, fusiform, lax, speltoid; glumes glabrous, brownish, long, narrow, distinctly keeled throughout entire length; shoulders narrow, oblique; beaks 2 to 10 mm. long; awns 3 to 8 mm. long; kernels red, long, hard.

In the course of these studies it was found that oftentimes the culture of leaf rust, as obtained from the field, produced two or more quite distinct types of infection on one or more of the varieties inoculated. In order to determine whether this represented a variation in the reaction of the variety or was due to a mixture of two or more physiologic forms, these varieties were again inoculated with the rust. If more than one form was present, there would be a rapid multiplication of the one to which the variety was susceptible, while there would be little or no multiplication of the strain to which the variety was resistant. The same set of varieties was again inoculated with the same rust and the results compared.

The results obtained from the study of a culture received from Mr. C. O. Johnston, of Manhattan, Kansas, well illustrates the condition found in field collections. This culture severely infected Malakoff, C. I. 4898, producing a type 4 infection; whereas Mediterranean, C. I. 3332, showed several necrotic spots containing occasionally a small uredinium. Associated with these, however, were a few well developed uredinia which were accompanied neither by necrosis nor chlorosis. Urediniospores from these well developed uredinia were placed on plants of Mediterranean, C. I. 3332, where they produced abundant infection of type 4. The rust as developed on Mediterranean was then used as inoculum on Malakoff, C. I. 4898, and Mediterranean, C. I. 3332. The latter showed a type 4 infection, while Malakoff showed only a slight flecking (type 0), no uredinia being developed. The original culture of rust was then sown on Malakoff, and the rust obtained from this variety was transferred to Malakoff and Mediterranean. As a result, Malakoff was heavily rusted, showing type 4 infection. Mediterranean, on the other hand, showed considerable necrosis with an occasional small uredinium (type 1). Apparently the original culture consisted of at least two forms, one much more prevalent than the other.

In a somewhat similar manner, a culture obtained from aecia of the leaf rust of wheat on *Thalictrum flavum*[5] L. produced heavy infection on Mala-

[5] The aecia from which this culture was made were obtained by inoculating *Thalictrum flavum* in the greenhouse with wintered teliospores of *Puccinia triticina* from Clemson College, S. C. Aeciospores were collected from a number of leaves of *Thalictrum* and transferred to wheat (Michigan Amber) from which uredinia subsequently were obtained. The culture thus developed on wheat was sown as indicated.

koff, C. I. 4898, and the spring wheats, C. I. 3756, and Webster, C. I. 3780. Each of these varieties was reinoculated with the same rust. The three cultures of rust thus established were each transferred back to the same three varieties. The culture carried on the variety Malakoff was then found to infect this variety heavily, while it produced only slight flecking on varieties C. I. 3756 and Webster. The culture carried on C. I. 3756 produced heavy infection on both C. I. 3756 and Webster, whereas Malakoff was only slightly flecked. The culture carried on Webster produced heavy infection on Webster and 3756, while Malakoff was only slightly flecked. In this case there was no indication in the original culture that more than one form existed, the two forms apparently being about equally prevalent in the mixture and infecting all three varieties so heavily that resistance as indicated by the slight flecking was obscured. The cultures thus isolated remained uniform for their actions on the varieties named above. Other examples might be given, but these are sufficient to show that mixtures of forms apparently often exist in cultures of leaf rust as obtained in the field.

While such mixtures may be immediately evident when cultured, as in the first example given above, in other cases, as in the second example, the mixture may not be suspected until after culturing and cross inoculating on a series of varieties. Attempts to isolate forms by such a method proved very unsatisfactory. In such mixtures, where varieties were susceptible to one form and only moderately resistant to the other, urediniospores might be produced by both, and the two forms of rust would still be continued when the rust was again used as inoculum. In these cases it was difficult to decide whether there really was a mixture or whether the results were due to the reaction of the variety to a single form of the rust. Even when two quite different cultures were separated, it was by no means certain that each of these might not also be a mixture of two or more physiologic forms. With the increasing number of varieties which could be used as differentials, the number of inoculations and cross inoculations necessary to purify cultures made this method prohibitive.

These experiences demonstrated that it was necessary to be sure that pure lines of the rust were being studied. So it was decided that a study of the physiologic forms present in the leaf rust of wheat must be based only on cultures which had been started from a single spore. The method used has been described above. During the winter of 1921–1922, fourteen cultures from single spores were obtained and studied on the varieties mentioned above which had previously given promise of being differential for the physiologic forms of the rust. The previous study of the rust cultures had indicated those which would most certainly produce distinct physiologic forms, and the cultures from single spores were made from these. This resulted in the finding of a number of distinct forms.

During the winter of 1921–1922, 129 varieties and strains of common wheat were studied for their susceptibility to from 4 to 12 single-spore cultures of leaf rust. These included most of the varieties which had been noted as less heavily rusted in the field as well as those noted as resistant in the greenhouse studies of previous years. Most of these proved to be susceptible to all the cultures used. In a few strains of Michigan Amber, Fultz, Fulcaster, Theiss, Red May, Beloglina, and Banat, a few outstandingly resistant seedlings occurred. These were grown to maturity and are giving promise of being valuable resistant lines of these varieties.

Several of the varieties which in previous years had shown marked resistance in the greenhouse were again highly resistant to part of the cultures in 1921–1922, except for a few susceptible seedlings. These were Mediterranean (Imperial Amber), C. I. 4860; Beloglina, C. I. 1544; Beloglina, C. I. 2239; Pacific Bluestem, C. I. 4821; Banat, C. I. 4879; and Hungarian, C. I. 4830. The reaction of Mediterranean, C. I. 5776 was very similar to that of Mediterranean, C. I. 3332 except for what was apparently due to a slight mixture by another variety. On account of these impurities, it was not considered desirable to continue to use these varieties in the study of physiologic forms until purified for rust resistance.

During the winter of 1921–1922, those varieties of the durums, emmers, spelts and einkorns which had previously shown some promise were studied for susceptibility to 12 single-spore cultures belonging to 10 different physiologic forms. Only two of the wheat varieties belonging to these groups, Einkorn, C. I. 2433, and Vernal emmer, S. D. 293, were comparable for resistance to the varieties listed in table 1. High resistance to certain cultures was noted in some of the varieties, especially Einkorn, C. I. 2433 and Vernal emmer, S. D. 293, the latter being resistant to some degree to all forms. In general, however, resistance was only moderate (type 2) and in many cases it was rather intermediate between types 2 and 3, well developed large and small uredinia being produced with and without either necrosis or chlorosis all on the same leaf, an infection comparable to the X type of Stakman and Levine (23). While high susceptibility to a number of the cultures was shown by some of the varieties, there were usually, although not always, some indications of chlorosis.

Since common bread wheats have been found which sharply differentiate the physiologic forms, the above varieties have not been used to any great extent in these studies. It is of interest to note, however, that while most of these varieties have been only moderately resistant or susceptible in these greenhouse studies, they have shown high resistance in the field. Observations during the period 1919 to 1924 inclusive, especially at LaFayette, Ind.; Washington, D. C.; Knoxville, Tenn.; Experiment, Ga.; and Madison, Wis., show that the varieties of durums, emmers, and einkorn listed above

have been highly resistant in the field, usually showing only flecking with the development of a few small uredinia. In a few instances, especially in the case of Khapli, there has developed 35 to 50 per cent of rust. In the seedling stage in the greenhouse, however, they have shown no such uniform rust resistance except in the two instances mentioned above.

Eleven varieties of common wheat showed marked resistance to some of the single-spore cultures. These are Malakoff, C. I. 4898; Turkey 47; Norka, C. I. 4377; Mediterranean, C. I. 3332; Hussar, C. I. 4843; Democrat, C. I. 3384; Webster, C. I. 3780; and the unnamed spring varieties, C. I. Nos. 3756, 3778, 3747 and 3779. By the use of these, the cultures of [illegible] rust easily could be separated, as they were for the most part either highly resistant or highly susceptible to the single-spore cultures. While a number of the other varieties mentioned could be used, these eleven give more clear-cut differentiations. As they are common wheats, the group in which leaf rust resistance is of the greatest importance, it was decided to use this set for the time being in a study of physiologic forms.

Some additional single-spore cultures were made and studied during the winters of 1922–1923 and 1923–1924, and two additional physiologic forms were distinguished and more information gathered concerning the others.

The susceptibility of the varieties employed, and the characteristics of the physiologic forms of the rust so far separated, are given in table 1. To designate the various physiologic forms differentiated, Arabic numerals are employed to conform to the usage adopted by the American Phytopathological Society in January, 1925.[6] The different forms may be separated by a key, which is given in table 2. Under the various physiologic forms in table 3 are listed the single-spore cultures according to the locality from which the original collection was obtained.

Each of these 33 single-spore cultures was sown at least two different times on 7 to 15 seedlings of each variety. Therefore at least 14 to 30 seedlings of each of the 11 varieties were inoculated with urediniospores from each culture. Certain of these cultures which have been continued for some time and have been used in other studies have furnished inoculum for a much greater number. Thus certain of the single-spore cultures belonging to physiologic forms 3, 5, and 9 have been transferred to each variety from 9 to 13 different times, involving a total of between 255 to 320 seedlings of each of the 11 differential varieties for each of these single-spore cultures.

No difficulty was experienced in obtaining infection and, except for a very few plants, accidental mixtures, all the seedlings showed the character-

[6] See Report of Joint Committee on Nomenclature. Phytopathology **15**: 316–317. 1925.

TABLE 1.—*Types of infection produced by twelve physiologic forms of the leaf rust of wheat, Puccinia triticina, on eleven differential strains of wheat*

Variety	Types of infection produced by physiologic forms											
	1	2	3	4	5	6	7	8	9	10	11	12
Malakoff, C. I. 4898	0	0-1	0-1	0-1	4	4	4	4	4	4	0	0
Turkey, 47	0	0-1	0-1	0-1	4	4	4	4	4	4	0	0
Norka, C. I. 4377	0	0-1	0-1	0-1	4	4	4	4	4	4	0	0
Unnamed, C. I. 3756	0	0-1	0-2	4	0	2	1-2	1	1-2	4	2+	4
do, C. I. 3778	0	0-1	2-2+[a]	4	0-1	4	1	4	1-2	4	3-4	4
Webster, C. I. 3780	0	0	0-2	1	0-1	1-2	4	4	4	4	1-2+	1
Unnamed, C. I. 3747	0	0	0-2	1-2	0-1	1	4	4	4	3-4	1-2+	0-1
do, C. I. 3779	0	0-1	2+[a]-4	3-4	0-1	3	1	4	4	4	3-4	4
Mediterranean, C. I. 3332	1	4	3-4	3-4	4	3-4	4	3-4	0-1	1-2	1-2	4
Hussar, C. I. 4843	1	3-4	0-1	2-2+	0-2+	3	1	1	1-2+	1-2	0-2	4
Democrat, C. I. 3384	0	4	4	1-2	4	3-4	4	1	0-1	1-2	0-2	4

[a] An infection type of 2+ is rather indeterminate. Uredinia are fairly abundant and large accompanied by pronounced chlorosis and sometimes some necrosis.

istic reaction of the variety as given in table 1 for the physiologic form in question. There was considerable difference between varieties in the amount of variation in reaction. Some varieties, such as Malakoff, Turkey 47, or Norka, showed very little. To physiologic forms to which these varieties were highly resistant, they showed uniformly a very high resistance under all conditions of inoculation. On the other hand, to some of the physiologic forms, some varieties, as for example Hussar, showed considerable variation, often being highly resistant when inoculated in the fall or winter and only moderately or slightly resistant when inoculated in late spring, indicating an environmental effect upon the reaction. These have been included in the range of susceptibility in table 1. With the study of additional cultures, it may be desirable to modify the characterizations of some of the physiologic forms to include cultures which may show but slight deviations. This is especially true of such forms as have been isolated but once and lost after but comparatively little study, including forms 1, 7, 8, and 12.

The reaction of these varieties of wheat to the physiologic forms is interesting because of the marked differences which were found to occur. The varieties Malakoff, C. I. 4898, Norka, C. I. 4377, and Turkey 47 sharply differentiate forms 1, 2, 3, 4, 11 and 12 from 5, 6, 7, 8, 9 and 10, being very highly resistant (types 0 or 1) to the first group and highly susceptible (type 4) to the second (Plate VII, A and C). These three varieties not only have agreed with each other in the type of infection produced by each of the 33 single-spore cultures belonging to the 12 physiologic forms, but also have reacted very similarly when inoculated with 118 bulk cultures obtained directly from the field. Malakoff is considered by Clark, Martin and Ball (3) to be synonymous with Turkey. This particular strain of Malakoff, bearing the C. I. accession number 4898, was derived from a single head selected by Dr. C. E. Leighty at the Kentucky Agricultural Experiment Station in 1913. Turkey 47 is a strain of Turkey obtained from the Kansas Agricultural Experiment Station. Apparently, it originated from a hybrid of Turkey with some other bearded wheat, as it was designated Turkey × Bearded. The strain, however, has all the characteristics of Turkey. "Norka, C. I. 4377, originated from a pure-line selection of a common wheat made from a plot of Kubanka durum wheat in 1908 by W. G. Shelley at Akron, Colo." The Turkey lines are hardy winter wheats with white glumes, while Norka is a spring variety with brown glumes. As far as the identification of physiologic forms of *P. triticina* so far studied is concerned, Malakoff, C. I. 4898, Turkey 47, and Norka, C. I. 4377, in reality constitute but a single differential group.

In this connection, it is of interest to note the susceptibility shown by a number of strains of Turkey (Plate IX, B). Five cultures of rust to

which Malakoff, C. I. 4898, is resistant, were transferred to another strain of Turkey received from the Kansas Agricultural Experiment Station under the name of Malakoff, Minn. 1479. This strain proved highly susceptible to all 5 of these cultures. In addition, 15 other strains of Turkey were inoculated with from 3 to 5 cultures of leaf rust to which Malakoff, C. I. 4898, is resistant. These were received under the names of Crimean, Minn. 832; Crimean, C. I. 1436; Hungarian, C. I. 5967; Kharkof, C. I. 4897; Kharkof, Minn. 1474; Kharkof, Tex. 583; Kharkof, Tex. 1500; Theiss, C. I. 4883; Theiss, C I. 5741; Turkey, Minn. 829; Turkey (Alberta Red), Minn. 1488; Turkey, Minn. 1548; Turkey, Minn. 1549; Turkey, Tex. 1497; and Turkey, C. I. 4884. All of these strains of Turkey proved more or less susceptible (type 3 or 4). They also were susceptible to a number of other cultures to which Malakoff, C. I. 4898, was susceptible. In fact, they were susceptible to all the cultures with which they were inoculated.

It is therefore evident that while two strains of Turkey—Malakoff, C. I. 4898, and Turkey 47—behave similarly, the variety Turkey is not uniform

TABLE 2.—*Key to the twelve physiologic forms of the leaf rust of wheat, Puccinia triticina*

Host and reaction	*Form No.*
Malakoff, C. I. 4898, resistant[a]	
C. I. 3756, resistant	
Mediterranean, C. I. 3332, resistant	1
Mediterranean, C. I. 3332, susceptible	
Hussar, C. I. 4843, susceptible	2
Hussar, C. I. 4843, resistant	3
C. I. 3756, susceptible[b]	
Mediterranean, C. I. 3332, resistant	11
Mediterranean, C. I. 3332, susceptible	
Democrat, C. I. 3384, susceptible	12
Democrat, C. I. 3384, resistant	4
Malakoff, C. I. 4898, susceptible[a]	
C. I. 3756, susceptible	10
C. I. 3756, resistant	
Mediterranean, C. I. 3332, resistant	9
Mediterranean, C. I. 3332, susceptible	
Webster, C. I. 3780, susceptible	
Democrat, C. I. 3384, susceptible	7
Democrat, C. I. 3384, resistant	8
Webster, C. I. 3780, resistant	
Hussar, C. I. 4843, resistant	5
Hussar, C. I. 4843, susceptible	6

[a] Types of infection 0, 1 and 2 are included here under the term resistant. Types of infection 3 and 4 are included under the term susceptible.

[b] Physiologic form 11 is included here, although C. I. 3756 shows a rather indeterminate type between 2 and 3. See table 1.

so far as its leaf rust susceptibility is concerned. It is also of interest that, although the strains of Turkey wheat are not similar in their behavior, a distinct variety, Norka, C. I. 4377, should agree so closely in its behavior with two of the strains of Turkey, Malakoff, C. I. 4898, and Turkey 47 (Plate VII, A and C, Plate IX, B, 2 and 3).

A somewhat similar situation exists in the variety Mediterranean. Mediterranean, C. I. 3332, is resistant to physiologic forms 1, 9, 10 and 11, and susceptible to the other eight (Plate VIII, B). This variety, however, is not so clear cut in its susceptibility to some forms as is Malakoff. When resistant, it may be only moderately so (type 2), as, for example, to forms 10 and 11; and oftentimes when it is susceptible to certain forms it is only moderately susceptible to them. While many uredinia are produced, they may be more or less accompanied by chlorosis as in the case of forms 3, 4, 6 and 8. Another strain of the variety Mediterranean, C. I. 5776, behaved very similarly to C. I. 3332. It apparently was not quite pure. Other strains of Mediterranean—C. I. 1395, C. I. 5614, and C. I. 5834—and Missouri Bluestem, C. I. 1912, showed themselves highly susceptible to cultures of the physiologic forms 1, 9 and 10 (Plate IX, A). It would appear, therefore, that within this agronomic variety there also exist different strains distinguishable by differences in their reaction to leaf rust.

Hussar, C. I. 4843, is resistant to 9 of the 12 forms, namely, 1, 3, 4, 5, 7, 8, 9, 10 and 11 (Plate VIII, C). Although it is resistant to a larger number of the physiologic forms than Malakoff, C. I. 4898, it is not always so highly resistant as the latter; the type of infection often approaches type 3, as in the case of forms 4, 5 and 9. On the other hand, this variety is often only moderately susceptible to the two forms, 2 and 6, and is most susceptible to form 12. Democrat, C. I. 3384, is resistant to six forms, 1, 4, 8, 9, 10 and 11 (Plate VII, D). It is also not quite so definite in some cases in its susceptibility and resistance as Malakoff.

The unnamed spring variety, C. I. 3756, is resistant to eight forms, 1, 2, 3, 5, 6, 7, 8, and 9. To form 11 it shows an intermediate reaction. To the remaining three forms, 4, 10, and 12, it is highly susceptible. The variety C. I. 3778 behaves very similarly to C. I. 3756 except that it is susceptible to forms 6 and 8 and more susceptible to forms 3 and 11 (Plate VIII, D). The spring variety, Webster, is resistant to eight forms, 1, 2, 3, 4, 5, 6, 11, and 12 (Plate VII, B). To four forms, 7, 8, 9, and 10, it is highly susceptible. The unnamed spring variety, C. I. 3747, has behaved very similarly to Webster, and the two may be considered as one differential group.

The unnamed spring variety, C. I. 3779, is resistant to four forms, 1, 2, 5 and 7. Its reaction to leaf rust, however, is not as pronounced in some instances as is the case with others of the differential varieties. Although

showing signs of resistance to form 3 (Plate III, A 1), in that some necrosis occurs, moderately large uredinia are produced in large numbers, many being accompanied by only a slight chlorosis. This differs from type 3 infection in the occurrence of necrotic areas and apparently resembles the indeterminate type of infection designated "X" by Stakman and Levine (23) for the stem rust of wheat.

TABLE 3.—*Localities from which the various physiologic forms of leaf rust of wheat have been obtained*

Physiologic forms	Number of cultures studied	Source and date
1	1	Pullman, Wash., 1921.
2	2	Murfreesboro, Tenn., 1921; Washington, D. C., 1923.
3	5	Manhattan, Kans., 1921, 1922; Jefferson, Ga., 1922; Washington, D. C., 1923; Experiment, Ga., 1924.
4	2	St. Paul, Minn., 1921; Pullman, Wash., 1921.
5	2	Jefferson Co., Kans., 1920; Knoxville, Tenn., 1922.
6	3	Madison, Wis., 1922, 1923; LaFayette, Ind., 1923.
7	1	Washington, D. C., 1921.
8	1	Murfreesboro, Tenn., 1921.
9	6	Raleigh, N. C., 1920; Ames, Iowa, 1921; Manhattan, Kans., 1922 (4 cultures).
10	8	Brookings, S. D., 1921; Jefferson Co., Kans., 1920; Madison, Wis., 1921; St. Paul, Minn., 1923; Bloomington, Ill., 1923; Lexington, Ky., 1921; Blacksburg, Va., 1921; Washington, D. C., 1923.
11	1	Berkeley, Calif., 1923.
12	1	Madison, Wis., 1922.

The order of the differential varieties, if listed according to the number of forms of rust to which they show resistance, would be: Hussar, C. I. 4843, resistant to nine; Webster, C. I. 3747 and C. I. 3756, each resistant to eight; Malakoff, C. I. 4898, Norka, C. I. 4377, Turkey 47, Democrat, C. I. 3387, and C. I. 3778, each resistant to six; C. I. 3779 and Mediterranean, C. I. 3332, each resistant to four. Malakoff, Norka, and Turkey 47 were outstanding for their uniform high resistance to all of the six forms to which they showed resistance.

Distribution of physiologic forms. Whether there is any regularity or fixity in the distribution of the physiologic forms is a matter of considerable importance, especially in connection with breeding for resistance. Most of our efforts have been directed towards discovering physiologic forms of the

rust and the varieties of wheat by which they may be differentiated. The time and labor involved in starting cultures from single spores, in order to make certain of purity of cultures, has limited the number of cultures which could be studied. While the 33 cultures thus studied cannot be considered as giving complete data on the distribution of the forms, taken with other data they afford some idea of the situation. Table 3 gives the localities from which the various physiologic forms of rust have been isolated. While form 10 is apparently the most prevalent, there is seemingly no sharp geographic limitation to the distribution of the various forms in the eastern United States. Too few single-spore cultures from the west have been studied to allow drawing any conclusions as to the apparent absence of such forms as 10 and 9 from that part of the country.

TABLE 4.—*Reaction of wheat varieties to original culture of leaf rust collected at Pullman, Washington, and to the single-spore cultures isolated from it*

Culture	Types of infection on										
	Malakoff C. I. 4898	Turkey 47	Norka C. I. 4377	C. I. 3756	C. I. 3778	Webster C. I. 3780	C. I. 3747	C. I. 3779	Mediterranean C. I. 3352	Hussar C. I. 4843	Democrat C. I. 3384
Original culture	0(4)[a]	0(4)[a]	0(4)[a]	4	4	3(0)[a]	1	4	0(4)[a]	1–2	0–1
Single-spore culture 1 (Physiologic form 1)	0	0	0	0	0	0	0	0	1	1	0
Single-spore culture 2 (Physiologic form 4)	0–1	1	1	4	4	1	2	3	4	2+	1

[a] A mixture of two types of infection, that represented by the number in parentheses being much less abundant.

Still further light is thrown upon this situation when the single-spore cultures are compared with the cultures from which they are isolated. In only a few cases, where both the original culture as obtained in the field and the single-spore cultures isolated and developed from it were studied, did they agree entirely in their behavior. This indicates that the physiologic forms of the leaf rust of wheat are quite generally mixed in the field.

While in most cases no attempt was made to isolate all the forms to be found at any one place, in a few cases a number of single-spore cultures were made which showed definitely the presence of several forms in the same locality. Thus from a collection of leaf rust made by Dr. H. B. Humphrey

at Pullman, Washington, on July 25, 1921, two single-spore cultures were obtained which differed markedly from each other, as is shown in table 4. It is to be noted, however, that the original culture not only showed evidence of this mixture of forms, but apparently a third physiologic form was present to which Malakoff was susceptible. This may have been form 10 and apparently made up only a small part of the mixture.

Another instance of mixture of physiologic forms has been demonstrated in the study of cultures from single spores isolated from a collection of rust received from Prof. L. E. Melchers from Jefferson Co., Kansas, in 1920. While the original culture was not studied on the full set of varieties, the results from those varieties inoculated showed evidence (table 5) that the original culture was a mixture. From this original culture two cultures were derived by continuing to transfer the rust to the varieties Malakoff and C. I. 3756. From each of the cultures thus developed, another was started from a single spore. The results of the study of these two cultures are given in table 5 and indicate that the original culture was a mixture of physiologic forms 5 and 10.

TABLE 5.—*Reaction of wheat varieties to original culture of leaf rust collected in Jefferson Co., Kansas, and to the single-spore cultures isolated from it*

Culture	Types of infection on										
	Malakoff C. I. 4898	Turkey 47	Norka C. I. 4377	C. I. 3756	C. I. 3778	Webster C. I. 3780	C. I. 3747	C. I. 3779	Mediterranean C. I. 3332	Hussar C. I. 4843	Democrat C. I. 3384
Original culture	4	...	...	0,4[a]	0,4[a]	0,4[a]	...	0,4[a]	...	...	...
Single-spore culture 1 (Physiologic form 5)	4	4	4	0	0–1	0	0–1	0	4	1	4
Single-spore culture 2 (Physiologic form 10)	4	4	4	4	4	4	4	4	2	1	2

[a] A mixture of types of reaction in about equal proportions.

In 1921, leaf rust, then being used by Mr. C. O. Johnston in his greenhouse studies at Manhattan, Kansas, was received for study. A culture from a single spore isolated from this material differed markedly from the original, as is shown in table 6. The next year Mr. Johnston again sent a culture of the leaf rust which he had been maintaining in the meantime in

the greenhouse. A study of this again gave nearly the same results except that Mediterranean and Democrat showed only a few well developed uredinia where there had been a moderate number before. A culture from a single spore (Culture 1) derived from the susceptible type on Mediterranean was developed and studied as well as four other cultures from single spores (Cultures 2–5) from the rust on Malakoff. The four latter all agreed

TABLE 6.—*Reaction of wheat varieties to original cultures of leaf rust collected at Manhattan, Kansas, and to single-spore cultures isolated from it*

Culture	Types of infection on										
	Malakoff C. I. 4898	Turkey 47	Norka C. I. 4377	C. I. 3756	C. I. 3778	Webster C. I. 3780	C. I. 3747	C. I. 3779	Mediterranean C. I. 3332	Hussar C. I. 4843	Democrat C. I. 3384
Original culture, 1921	4	4	4	2	2	4	4	4	4,1[a]	3	4,0[a]
Single-spore culture Physiologic form 3	1	1	1	2	2	1	1	4	3	1	4
Original culture, 1922	4	4	4	2	2	3–4	3–4	4	1(4)[b]	3	0(4)[b]
Single-spore culture 1 Physiologic form 3	0	0–1	0–1	1	2	0	0–1	2+	4	0–1	4
Single-spore culture 2 Physiologic form 9	4	4	4	2	2	4	4	4	0	2+	0–1
Single-spore culture 3 Physiologic form 9	4	4	4	2	2	4	4	4	1	2+	1
Single-spore culture 4 Physiologic form 9	4	4	4	2	2	4	4	4	1	2+	1
Single-spore culture 5 Physiologic form 9	4	4	4	2	2	4	4	4	1	2+	0–1

[a] A mixture of two types of infection in about equal proportions.

[b] A mixture of two types of infection, that represented by the number in parentheses being much less abundant.

very closely with each other, proving to be physiologic form 9, but differed markedly from that obtained from Mediterranean, which proved to be form 3. The latter agreed very closely with the single-spore culture obtained in 1921. Apparently Mr. Johnston's original culture consisted of a mixture of forms 3 and 9, probably in nearly the same proportion. After being carried

for a year in the greenhouse, form 3 apparently became reduced and made up only a small part of the mixture.

While it has been impossible to study more cultures derived from single spores, some additional evidence as to the mixture of physiologic forms has been accumulated. Some data obtained in the earlier part of the study and which made evident the necessity of deriving cultures from single spores already have been partly discussed. In addition to this, rust was collected in 1923 on a number of the differential varieties in several different nurseries to determine if the rust in the different localities was homogeneous. The results of this study are given in table 7. It would appear that the rust in all three localities was not uniform. At least two and probably more physiologic forms were present at each place. It is interesting to note that, both at Knoxville and LaFayette, the rust from Mediterranean, C. I. 3332, did not infect Malakoff when inoculated in the greenhouse, while the rust from Malakoff infected both hosts. Apparently, also, the rust on the varieties C. I. 3747 and Webster at LaFayette was not well adapted to these varieties, for they proved highly resistant when inoculated with the rust from the same variety in the field.

There is some evidence that the physiologic forms of leaf rust present in any given locality may vary from year to year. While it has not been possible to accurately determine the exact identity of these forms from studies of single-spore cultures, a study of the susceptibility of varieties in the field for several years and testing of the rust cultures in the greenhouse indicate that such variations occur. No attempt will be made here to give all the data, a few examples being sufficient.

The variety Malakoff showed only a trace to 5 per cent of rust in the varietal nursery at LaFayette in 1919. To cultures of the rust collected that summer and studied during the winter in the greenhouse, Malakoff showed only a slight flecking, agreeing with the field evidence and indicating that physiologic forms of leaf rust to which Malakoff is susceptible were either scarce or lacking at LaFayette in 1919. In 1920, Malakoff showed 50 to 65 per cent of rust in nurseries; and studies conducted during the following winter in the greenhouse indicated that two physiologic forms were present, this variety being highly susceptible to one culture and resistant to the other. While not especially studied in the greenhouse the two following years, the host reaction in the field and the results shown in table 7 for 1923 indicate that two or more physiologic forms were again present, Malakoff being susceptible to at least one form and resistant to another.

There is also some evidence that, while physiologic forms to which Malakoff is susceptible may be present over a wide territory (table 3), they

develop best in the hard winter and spring wheat areas of the United States, where they are more frequently isolated than in the south and east. Thus in the territory comprising the States of New York, Pennsylvania, District of Columbia, Virginia, North Carolina, South Carolina, Georgia, Alabama, West Virginia and Mississippi, 29 collections of leaf rust have produced no infection on Malakoff, 23 produced heavy infection, and 9 were very evident mixtures. In the territory comprising the States of Tennessee, Kentucky, Indiana, Michigan and Illinois, only 7 collections produced no infection on Malakoff, 28 infected that variety heavily and 7 were evident mixtures. In the territory including the States of Wisconsin, Minnesota, South Dakota, Iowa, Kansas, Oklahoma, Arkansas and Missouri, only 3 collections pro-

TABLE 7.—*Reaction on wheat varieties caused by cultures of the leaf rust of wheat obtained from different varieties in the field*

Location of Varietal Nursery	Variety of wheat from which culture was obtained	Percentage[a] of field susceptibility of variety from which culture was obtained	Types of infection on					
			Malakoff C. I. 4898	C. I. 3756	Webster C. I. 3780	C. I. 3779	Mediterranean C. I. 3332	Democrat C. I. 3384
Washington, D. C.	C. I. 3756	100	0	4	1	4, 1[b]	4	4
	Webster C. I. 3780	50	0	4	2	4	4	4
	Mediterranean C. I. 4860	100	4	4	0	4	4, 1[b]	1
Knoxville, Tenn.	Malakoff C. I. 4898	trace—25	4	3	0	4	4	2, 4[b]
	Norka C. I. 4377	50	4	3	0	4	4	4
	Mediterranean C. I. 3332	100	0	0	0	4	4	4
LaFayette, Ind.	Malakoff C. I. 4898	35–65	4	1	1	2	4, 1	
	C. I. 3756	75–100	4, 1[b]	3	1	4	4	
	Webster C. I. 3780	5–25	3	1(4)[c]	1	3	4	
	C. I. 3747	5–25	3	1	1	4	3, 1[b]	
	Mediterranean C. I. 3332	75–100	0	2	0	3	4	
	Hussar C. I. 4843	25–50	3(1)[c]	1	0	4	4	4

[a] Based upon scale used in the Office of Cereal Investigations.

[b] Approximately equal mixture of types indicating mixture of physiologic forms.

[c] Mixture of types, that in parentheses being less abundant, indicating unequal mixture of physiologic forms.

duced no infection on Malakoff, 22 infected that variety heavily, and 5 were evident mixtures. From Arizona, Utah, Nevada, Montana, Idaho, Washington, Oregon and California, 11 collections caused no infection on Malakoff, only one infected Malakoff heavily, whereas 3 evidently were mixtures. Although physiologic forms to which Malakoff is resistant occur in the second and third groups of states, yet it is apparently in this region that those forms to which Malakoff is susceptible are the most prevalent, decreasing eastward, southward, and especially westward.

DISCUSSION

The results of this investigation show that the leaf rust of wheat consists of a number of physiologic forms distinguished by differences in their ability to parasitize varieties of wheat. A situation thus exists in this rust similar to that reported by Stakman and Levine (23) for the stem rust of wheat. In both cases these physiologic forms show but little morphologic basis for distinctions and are best identified by their effect when transferred to certain varieties of wheat.

Twelve physiologic forms of the leaf rust of wheat have been isolated thus far and distinguished by using 11 strains of wheat, comprising eight differential groups. Several of these forms of rust differ markedly from each other. For example, while all the eleven varieties are resistant to form 1, only three are resistant to form 10. Vernal emmer, S. D. 293, is the most outstanding of the varieties for resistance in the seedling stage in greenhouse studies, being more or less resistant to all of the cultures sown upon it. Unfortunately no variety of bread wheat has been found resistant to all of the physiologic forms of leaf rust, although several were resistant to eight of the twelve forms.

With the exception of Vernal emmer, S. D. 293, no other variety has been found resistant in the seedling stage to all the physiologic forms of leaf rust of wheat in these studies. On the other hand, no physiologic form of leaf rust has been discovered so far to which several varieties or strains of wheat have not been found resistant. In view of the results obtained by Hayes and Aamodt (10), Harrington and Aamodt (9), and Puttick (21), in connection with breeding wheat for stem rust resistance, it seemed very probable that wheats can be made still more generally resistant to leaf rust by selecting as parents of crosses those varieties which will combine resistance to the largest number of physiologic forms of leaf rust. Studies now being conducted with such crosses indicate that resistance to leaf rust may be combined in a similar manner. It should be emphasized, however, that a number of varieties, Kanred among the common bread wheats and especially certain varieties of the durums, emmers, and einkorns have gen-

erally exhibited a remarkably high resistance in field sowings. Most of these, however, under greenhouse conditions have not shown a reaction to all the physiologic forms at all comparable to their reaction under field conditions. Although many of the durums, emmers, and einkorns can be used as differential varieties, their susceptibility to a number of the forms is not so clear as is that of those varieties which have been selected and used for that purpose.

It is evident that what we in general call resistance to leaf rust of wheat is not due to one factor, but is caused by a number of different factors. Thus the high resistance shown by Malakoff, C. I. 4898, Turkey 47, and Norka, C. I. 4377 to six of the twelve physiologic forms of leaf rust, namely, 1, 2, 3, 4, 11 and 12, to all appearances is very similar to that shown by Mediterranean, C. I. 3332. The latter, however, is susceptible to physiologic forms 2, 3, 4 and 12, but resistant to 9 and 10, to which the first three varieties are susceptible. Other varieties show still other manifestations of this condition. Again, while some varieties like Malakoff possess a resistance which apparently is not easily changed, others, like Kanred and a number of durum and emmer varieties, may show a much higher resistance in the field than they do when grown in the greenhouse.

The resistance found in the strains of wheat, Malakoff, C. I. 4898, Turkey 47, and Norka, C. I. 4377 presents an interesting situation. These strains have all behaved very similarly, indicating that their rust resistance is likely to be due to the same factor or factors. The first two are considered by Clark, Martin, and Ball (3) as synonymous with Turkey, a white chaffed winter wheat. Norka is a brown chaffed spring wheat. While agreeing with Norka in reaction to leaf rust, Malakoff, C. I. 4898, and Turkey 47 differ markedly from a number of other lines of Turkey. In a similar way, Mediterranean, C. I. 3332, differs in its reaction to leaf rust from a number of other strains of Mediterranean wheat.

As the various strains of Turkey are grouped together in that variety largely because they possess in common such morphologic characters as winter habit, a white weak stem, awned spike, white glabrous glumes and hard red kernels, the lack of uniformity for rust resistance among such strains would indicate that the leaf-rust resistance of Malakoff, C. I. 4898, is not likely to be linked with any of these characters. For the same reason, the leaf-rust resistance of Mediterranean, C. I. 3332, is not likely to be linked with such characters as winter habit, purple stem, glabrous brown glumes, bearded spike, and soft red kernels.

From the above, it evidently is unsafe to draw general conclusions concerning agronomic varieties of wheat from the results obtained by the study of a single strain of the variety. If comparable results are desired in the

study of physiologic forms of leaf rust, the same pure-line resistant strain of the variety must be used by the various investigators. As resistance in most instances apparently is a physiologic character, or a combination of several such characters, rust-resistant lines of wheat can be distinguished with certainty only through their reaction to the rust. The rust and host are interacting organisms, and their reactions serve to characterize each other.

The twelve physiologic forms of leaf rust have been studied hardly long enough to justify any definite conclusion as to their fixity. The accumulated evidence, however, indicates that there is no very great change in their ability to infect varieties beyond a slight fluctuation, due probably to the effect of changing environmental conditions on the host.

The physiologic forms of the leaf rust of wheat apparently are very seldom found pure in any locality, but usually several are found mixed together. This is not surprising, as the leaf rust organism is a wind-blown pathogene. Wheat is grown quite generally throughout the eastern and central United States, and leaf rust is very common in this territory every year. The studies of Stakman et al (22) on the spore content of the upper air show that leaf rust urediniospores are found up to an altitude of 10,000 feet or higher. This indicates that spores may be blown long distances from their point of origin. When we consider that wheat is usually quite generally infected with leaf rust, and that wheat is so commonly planted, in some localities almost in continuous areas, it is not surprising that there is more or less mixture of physiologic forms of rust.

It is possible, however, that different climatic conditions in various parts of the United States may impose some limitations on the prevalence of the different physiologic forms. It is conceivable that the physiologic forms may differ in respects other than in ability to infect certain varieties of wheat. They may differ in the range of temperatures through which their spores will germinate, in ability to develop in the host under various climatic conditions, and in ability to survive the winter in the host tissue. The studies of Hursh (12) and Peltier (20) with physiologic forms of the stem rust of wheat have shown that these differ somewhat in ability to germinate and infect under some of these conditions. The varieties of wheat grown in any given region also undoubtedly play some part. While these factors may not entirely prevent physiologic forms from developing, they may play a considerable part in determining the relative prevalence of the forms.

It is evident, however, that both the existence of physiologic forms, with their differences in ability to parasitize varieties, and the apparent fluctuations in their distribution complicate exceedingly the problem of breeding wheat varieties resistant to leaf rust. This situation makes desirable a complete knowledge of the physiologic specialization in the rust and a

knowledge of the susceptibility of wheat varieties to the various physiologic forms. It is desirable that, if possible, some clew be obtained concerning the origin of such forms, or at least, whether new forms are being originated frequently. This investigation therefore offers no hope of immediate, complete solution.

The results here recorded can be considered only as merely introducing the problem. This study can be considered only as a preliminary survey of the question of specialization in the leaf rust of wheat. Other varieties and strains of wheat will be discovered, possessing resistance to physiologic forms of leaf rust. Such additional differential varieties will make possible the identification of forms which can not now be distinguished. With Malakoff the only differential variety, only two forms of rust could be distinguished; by the use of C. I. 3756 in addition, four forms were recognized.

It is necessary, also, to recognize that some of the varieties which have been discarded because of their susceptibility to all the cultures used in these investigations may show resistance to physiologic forms which have not yet been isolated in a pure condition. For example, it is very likely that Malakoff would have been discarded if the cultures obtained in the beginning of this work had been restricted to the great-plains region where physiologic forms of the leaf rust to which Malakoff is susceptible apparently predominate. The method followed in these studies of using cultures of rust from as many sections as possible, and the study of varieties in nurseries in various localities, it is believed, reduce the possibility of overlooking resistant varieties.

It must also be recognized that the information on susceptibility has been obtained on seedlings of the various varieties grown in the greenhouse. It does not follow that the reaction in the field will be identical. In fact, as stated, varieties which have shown promising resistance in the field have been more or less susceptible in the greenhouse studies. Such varieties are not being discarded but are being studied in the nurseries, the reason for the apparent difference in behavior being an important question for future investigation. While it is by no means definitely established, from the information at hand, it would appear that varieties resistant in the seedling stage in the greenhouse are likely to be fully as resistant or even more resistant to the same physiologic form in the later stages of development of the wheat plant in the field.

NOMENCLATURE OF LEAF RUSTS

It may be well to discuss here briefly the nomenclature which has been used in connection with the leaf rust of wheat. While the whole question of nomenclature of physiologic forms is too unsettled to attempt to draw final conclusions in regard to the name which should be used for this rust, yet a brief discussion of the situation with respect to this particular rust

and a few comparisons seem desirable. When Eriksson and Henning (7) separated *Puccinia glumarum* from the leaf rusts of the cereals and wild grasses, they proposed the name *Puccinia dispersa* for the remainder of the leaf rusts on wheat, rye, and wild grasses. Eriksson (5), as the result of further studies, recognized four specialized forms or varieties in this species: 1. *secalis* on *Secale cereale* with aecia on *Anchusa* spp.; 2. *tritici* on *Triticum vulgare;* 3. *bromi* on *Bromus arvensis* and *B. brizaeformis;* and 4. *agropyri* on *Agropyron repens,* the last three with aecia unknown at that time.

As the result of still further study, Eriksson (6) found that each of these forms was very closely specialized to a few hosts and considered that they were therefore worthy of being considered as species. He retained the name *Puccinia dispersa* for the form on rye. The leaf rust of wheat was named *Puccinia triticina;* that on species of Bromus, *Puccinia bromina;* that on *Agropyron repens, Puccinia agropyrina;* that on *Holcus lanatus* and *H. mollis, Puccinia holcina;* and that on *Trisetum flavescens, Puccinia triseti.* It is to be noted that these rusts are to be distinguished largely by physiological differences, host specialization, time of spore germination, etc., as only minor morphologic differences occur.

Since these studies of Eriksson were published, a number of investigators have extended our knowledge of the host specialization of this group and have demonstrated the aecial connection of several additional forms with different species of the families Boraginaceae, Hydrophyllaceae, Ranunculaceae, and Balsaminaceae. With one or two exceptions, there is a more or less close specialization to a few species of hosts, while the morphology is very similar. A number of investigators, following Eriksson's lead, have considered these specialized forms of rust worthy of specific rank and have given names to them. The writers (13) have discussed a number of those forms which have aecia on species of *Thalictrum,* but a fairly large number of other genera of the Ranunculaceae, as well as Boraginaceae, Hydrophyllaceae, and Balsaminaceae are known to include aecial hosts for the grass rusts of this group.

Some investigators, however, have not considered all of these forms worthy of specific rank. Thus Arthur and Fromme (1) list a number of such forms, including the leaf rust of wheat, having aecia upon different species of the family Ranunculaceae, as belonging to one species, *Dicaeoma clematidis* (DC.) Arth. Cunningham (4), concurring in this view but objecting to this name on the basis that it was a name first applied to the aecia and not tenable according to the International Rules of Nomenclature, considers that the name *Puccinia elymi* Westnd. should be used. Forms having aecia on species of the families Boraginaceae (*Puccinia dispersa, P. bromina*), Hydrophyllaceae (*Puccinia apocrypta* Ellis & Tracy), and Balsaminaceae (*Puccinia impatientis* (Schw.) Arth.), however, do not differ

morphologically from those with aecia on species of the Ranunculaceae more than the latter differ among themselves. In consequence, if species are to be founded entirely or largely on a morphological basis, it would seem that these also should be included. We would then have the interesting situation that the species as thus conceived would be at least very similar to Eriksson's original conception of the species *Puccinia dispersa*. However, it would be more desirable to use the name *Puccinia rubigo-vera* (DC.) Wint. for this species as thus conceived, because the name *Puccinia dispersa* has become established in the literature as applying only to the race of leaf rust found on rye. If aecial names are used, the name would be *Puccinia asperifolii* (Pers.) Wettst. There would be much less confusion involved in using the name *Puccinia rubigo-vera*, which was more or less extensively used for this group of rusts until superseded by the names proposed by Eriksson for the different races.

If the leaf rusts of rye, wheat, and wild grasses, with the general morphology of the leaf rust of wheat, are considered as one species, the situation would be more nearly comparable to that of the stem rust of wheat, *Puccinia graminis*, especially if the stem rust of timothy, with its aecia unknown, is considered as belonging to the latter species. The leaf rust species would then consist of a large number of races which, however, would be distinguished not only by the grass host to which they are specialized, but unlike *Puccinia graminis*,[7] also by the aecial hosts to which they are specialized. Upon this basis the race of leaf rust of rye would then be *Puccinia rubigo-vera dispersa* (or *secalis*); that of *Agropyron repens*, *Puccinia rubigo-vera agropyrina;* that of wheat, *Puccinia rubigo-vera triticina* (or *tritici*), with suitable names for other grass races. With this concept, the physiologic forms of the leaf rust of wheat would be denoted in a way similar to that employed for the physiologic forms of stem rust. Thus form 1 would be *Puccinia rubigo-vera triticina* p. f. 1, and the others would be designated similarly.

The writers believe that such a treatment of these leaf rusts is desirable, as it brings out most clearly the basis upon which they are distinguished. However, it is somewhat doubtful whether, because of the general use in the literature of such names as *Puccinia dispersa* for the leaf rust of rye, and *Puccinia triticina* for the leaf rust of wheat, such a change might not be more confusing than clarifying. It is largely for this reason that the name *Puccinia triticina* has been used in this paper. It should be kept in mind, however, that, if binomials are retained for these rusts, they apply to groups of rusts which can be distinguished from groups of similar morphology very largely by their physiologic specialization to certain hosts,

[7] The stem rust of timothy with its aecial host unknown but not producing aecia on *Berberis vulgaris*, the aecial host for the rest of the species, may be an exception.

and therefore are comparable to races or varieties in such species as *Puccinia graminis.*

SUMMARY

1. The leaf rust of wheat, *Puccinia triticina,* consists of at least twelve physiologic forms distinguished by the manner in which they infect eleven selected differential strains of wheat.

2. Twenty-five strains of wheat have been found to be more or less resistant to one or more of these forms. These are Malakoff, C. I. 4898; Turkey 47; Norka, C. I. 4377; Mediterranean, C. I. 3332; Mediterranean, C. I. 5776; Mediterranean (Imperial Amber), C. I. 4860; Hussar, C. I. 4843; Beloglina, C. I. 1544; Beloglina, C. I. 2239; Pacific Bluestem, C. I. 4821; Webster, C. I. 3780; unnamed spring wheats, C. I. Nos. 3756, 3778, 3747, and 3779; Peliss, C. I. 1584; Acme, S. D. 284; Arnautka, Minn. 337; Monad, C. I. 3330; Mindum, C. I. 5296; Khapli emmer, C. I. 4013; Vernal emmer, S. D. 293; Double einkorn; and Einkorn, C. I. 2433.

3. Of these, eleven varieties, comprising eight differential groups, have been found to distinguish best the twelve physiologic forms of the rust. These are Malakoff, C. I. 4898; Turkey 47; Norka, C. I. 4377; Mediterranean, C. I. 3332; Hussar, C. I. 4843; Democrat, C. I. 3384; Webster, C. I. 3780; and the unnamed spring varieties, C. I. Nos. 3756, 3778, 3747, and 3779.

4. The physiologic forms of the rust are distinguished by the reaction shown by these varieties when infected with leaf rust. No form has been found so far to which all of these varieties are susceptible. On the other hand, other than the variety Vernal emmer, S. D. 293, no variety has been found which in the seedling stage in the greenhouse has proved highly resistant to all twelve of these forms.

5. The physiologic forms of the leaf rust of wheat are not generally found pure in the field, but practically always several are found mixed together.

6. In order to study and identify such forms, it is necessary to obtain them in a pure condition. For this purpose, pure lines of the rust have been developed by starting each culture from a single urediniospore.

7. Physiologic forms of the rust are neither fixed nor limited in their distribution. Apparently those forms to which Malakoff, C. I. 4898, is susceptible are the most prevalent in the central west, while those to which this variety is resistant are more common in the east and south.

8. Agronomic varieties of wheat are not necessarily uniform in their reaction to leaf rust. Different strains of such varieties may differ markedly in their reaction.

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PLATE VI

Types of reaction shown by wheat to leaf rust, *Puccinia triticina*. ×3.

0. Highly resistant; no uredinia formed; flecked.

1. Very resistant; more or less flecked, accompanied by few small uredinia.

2. Moderately resistant; uredinia fairly abundant and of moderate size, accompanied by necrosis.

3. Moderately susceptible; uredinia fairly abundant and of moderate size, sometimes with chlorosis.

4. Very susceptible; uredinia abundant, large, sometimes in more or less pronounced green islands.

Upper row from left to right: (0) Norka by form 12; (0) Malakoff by form 1; (1) Malakoff by form 3; (1) C. I. 3780 by form 3; (2) Peliss by form 9; (2) C. I. 3756 by form 9.

Lower row from left to right: (3) Arnautka, Minn. 337 by form 5; (3) Imperial Amber by form 5; (4) C. I. 3769 by form 9; (4) C. I. 3756 by form 12 (compare under 2); (4) Norka by form 9 (compare under 0); C. I. 3747 by form 9.

PLATE VII

Comparison of four differential varieties of wheat for reaction to three physiologic forms of leaf rust, *Puccinia triticina*.

	1. Form 3	2. Form 9	3. Form 11
A. Norka, C. I. 4377	resistant	susceptible	resistant
B. Webster, C. I. 3780	resistant	susceptible	moderately resistant
C. Malakoff, C. I. 4898	resistant	susceptible	resistant
D. Democrat, C. I. 3384	susceptible	resistant	resistant

Note the close agreement of Norka (A) and Malakoff (C).

PLATE VIII

Comparison of four differential varieties of wheat for reaction to three physiologic forms of leaf rust, *Puccinia triticina*.

	1. Form 3	2. Form 9	3. Form 11
A. C. I. 3779	moderately susceptible	susceptible	susceptible
B. Mediterranean, C. I. 3332	moderately susceptible	resistant	resistant
C. Hussar, C. I. 4843	resistant	moderately resistant	resistant
D. C. I. 3778	moderately resistant	moderately resistant	susceptible

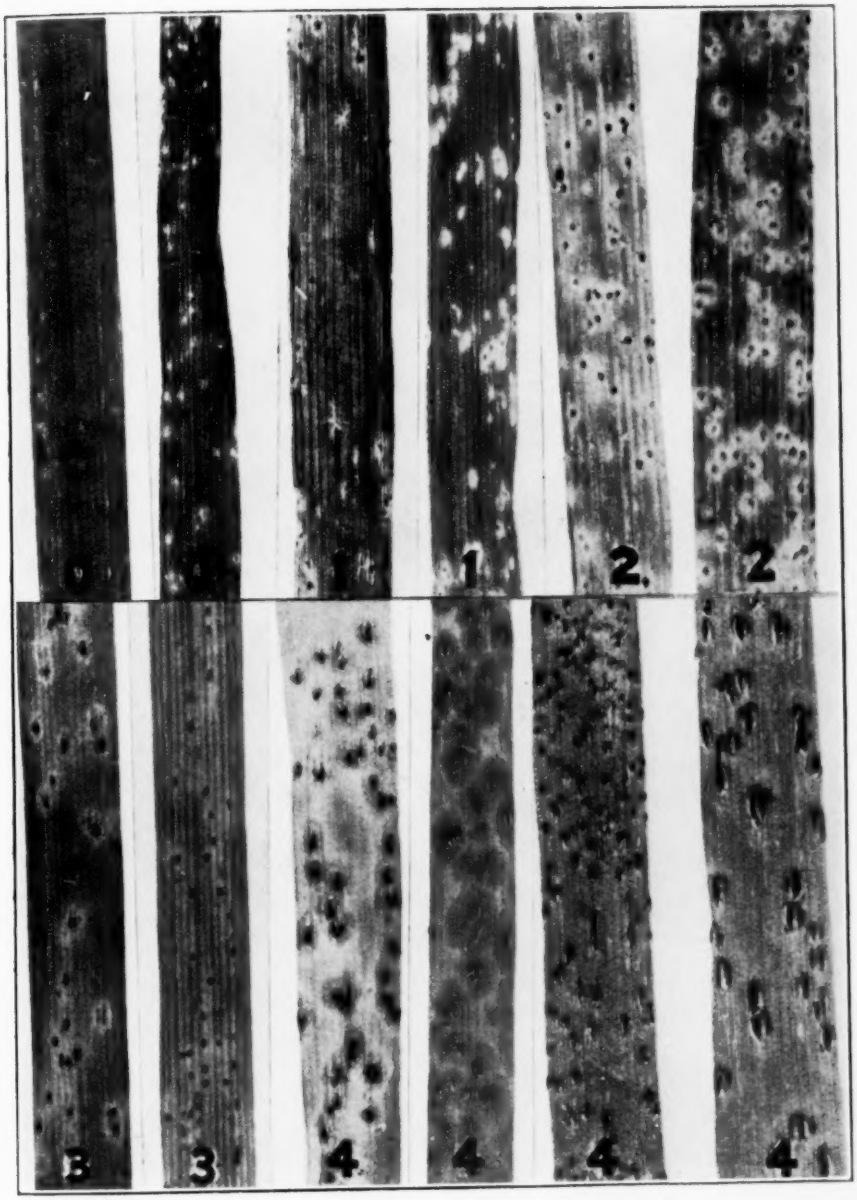
PLATE IX

Difference in reaction to the same physiologic form, I, of leaf rust, *Puccinia triticina*, shown by different strains of the same variety.

A. Variety Mediterranean; Resistant, (1) strain C. I. 3332, (2) strain C. I. 5776; Susceptible, (3) strain C. I. 5834, (4) strain C. I. 1395, (5) strain C. I. 5614.

B. Variety Turkey; Resistant, (2) Malakoff, C. I. 4898, (3) Turkey, strain 47; Susceptible, (4) Malakoff, Minn. 1479, (5) Turkey strain Minn. 1548, (6) Turkey strain Minn. 1549, (7) Kharkoff, C. I. 4897.

B1. Norka, C. I. 4377, agreeing in resistance with the Malakoff, C. I. 4898, and Turkey 47, strains of Turkey.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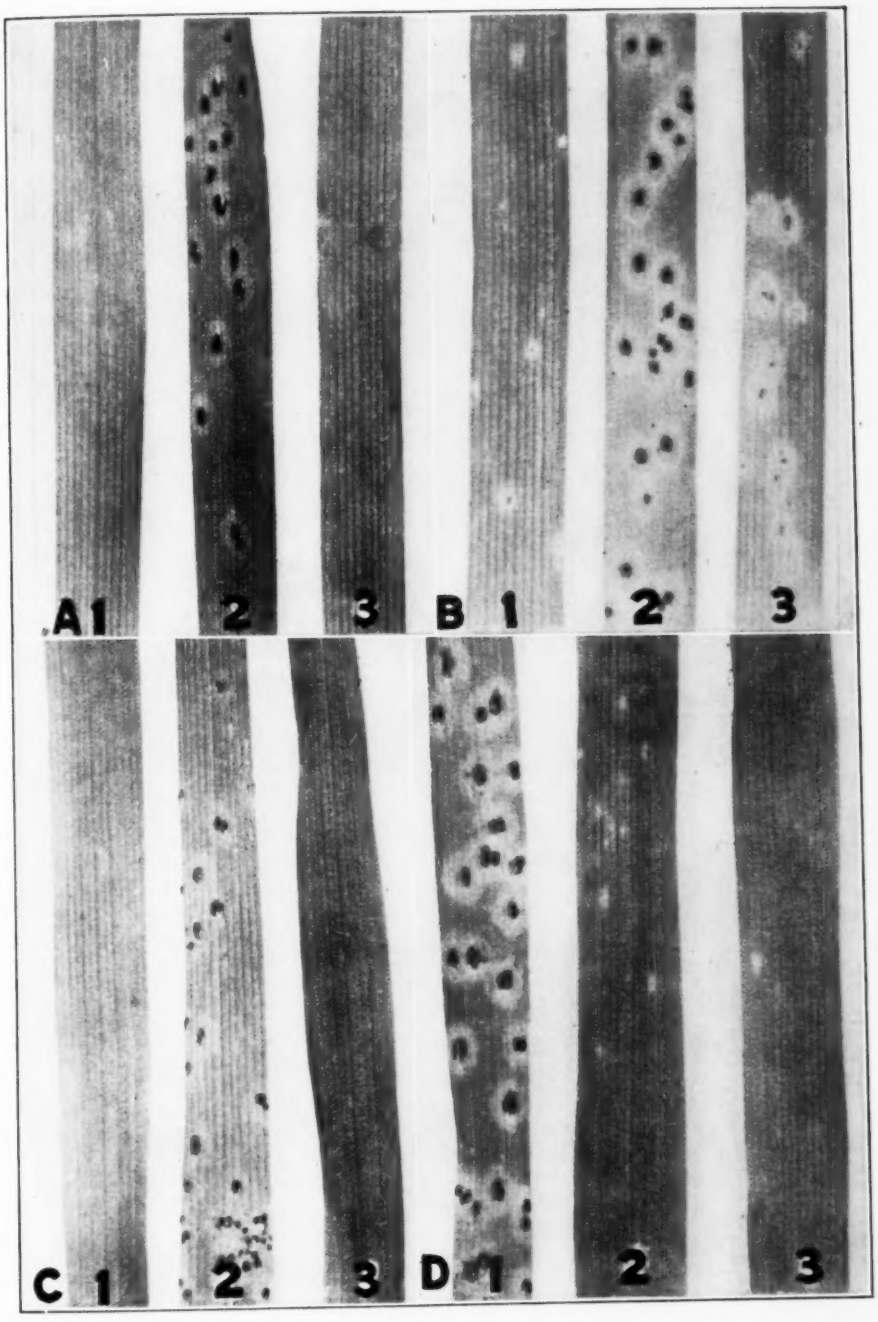
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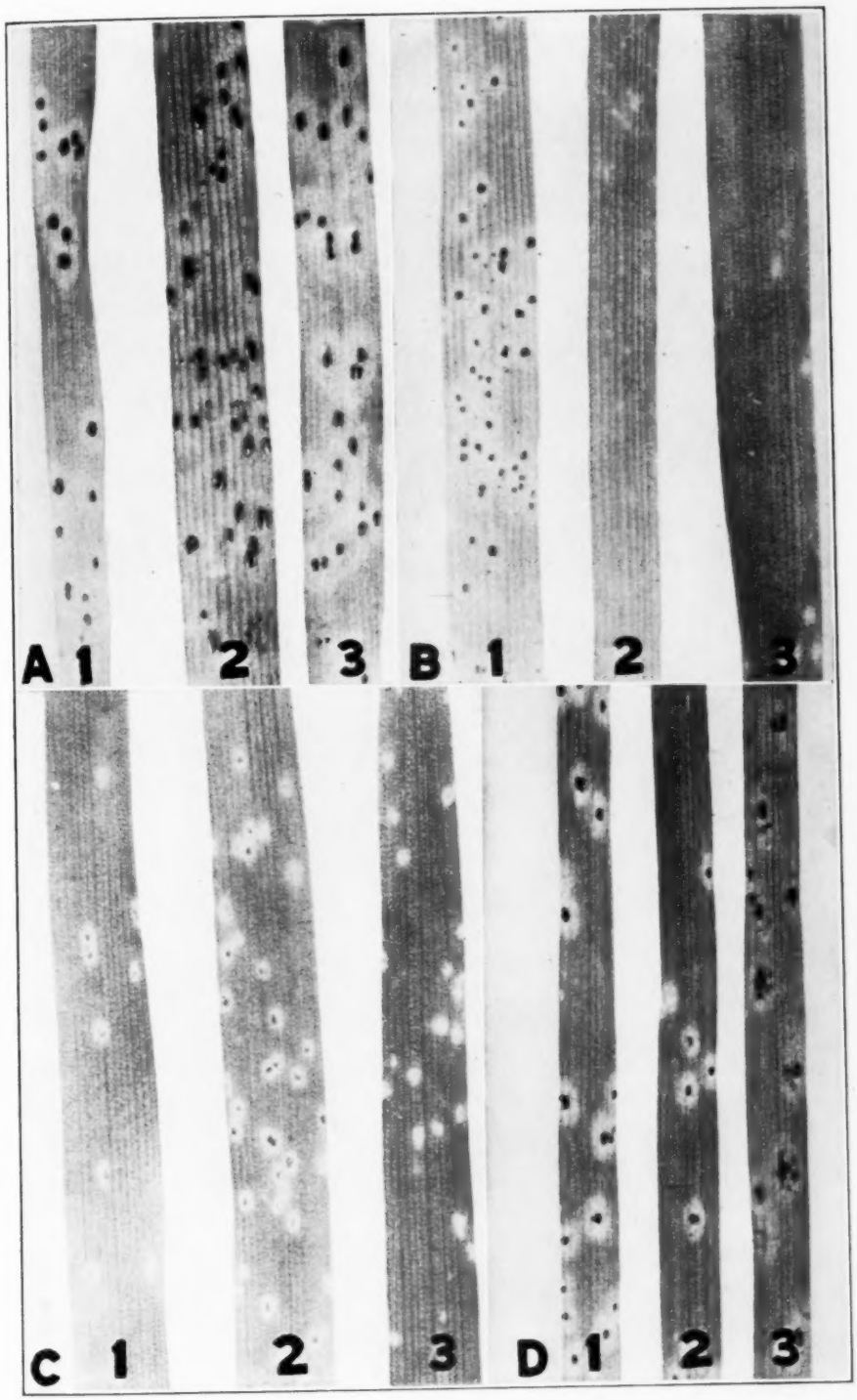
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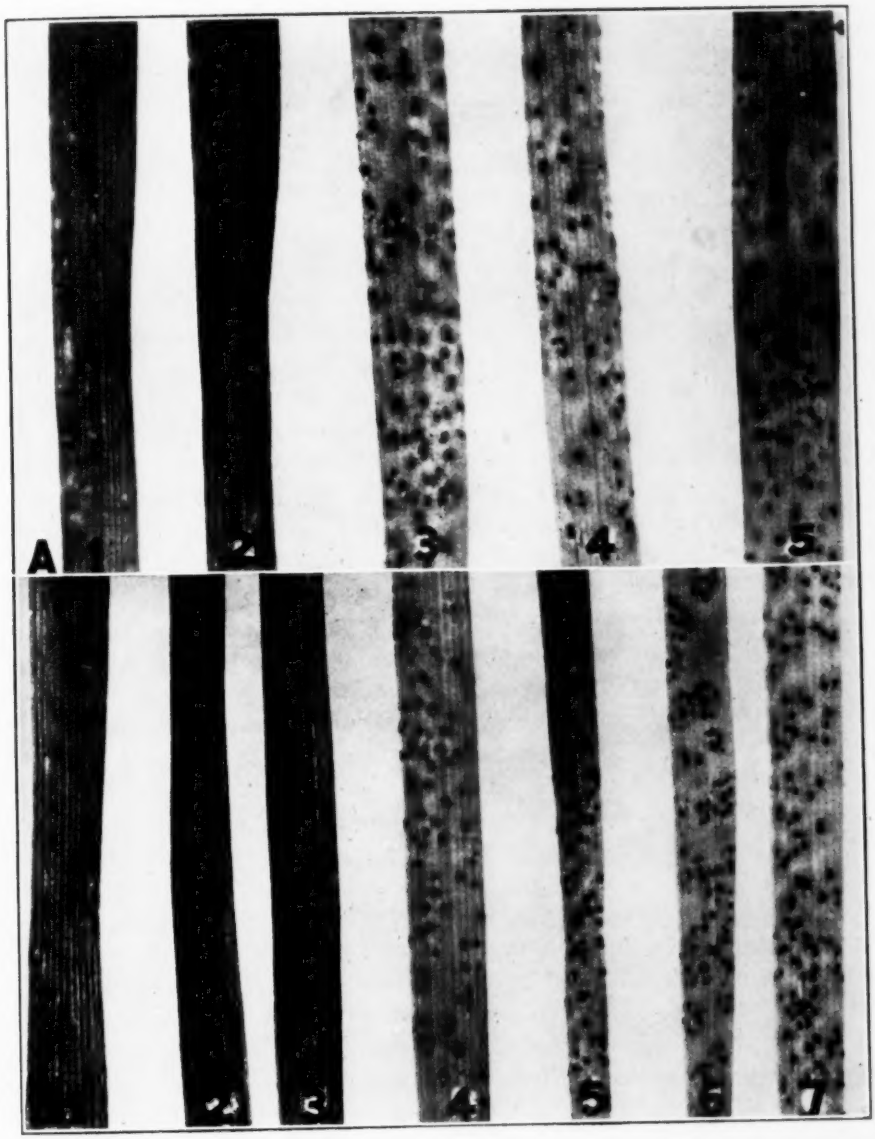
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THE OVERWINTERING OF PHYTOPHTHORA INFESTANS (MONT.) DE BY.

HELENA L. G. DE BRUYN

WITH THREE FIGURES IN THE TEXT

INTRODUCTION

The overwintering of *Phytophthora infestans*, the potato blight fungus, is still an unsolved problem. Since De Bary's idea (2, 3, 4) that the surviving mycelium in the diseased potato tuber is the only mode of hibernation of the fungus, numerous efforts have been made to settle this question experimentally, but no definite, convincing results have been obtained. Many observed facts still point to the inadmissibility of De Bary's opinion, and suggest other explanations for the overwintering of the fungus. The most satisfactory one is that of the hibernation of the fungus in the field; and as long as no striking results are obtained to show that this is impossible, it may be looked upon as explaining the observed facts far better than the theory that the diseased tuber is the only way by which the fungus remains alive. This does not mean, however, that mycelium hibernating in the tuber may never be the source of infection of the foliage of the succeeding year's crop. It is quite possible that under special circumstances the diseased tuber may be, in some way or other, the origin of the outbreak of the disease. All outbreaks, however, cannot be explained in this way, *e.g.*, those starting very late in the season during dry summers. The first thing to esablish is whether overwintering in the field can occur, and then to determine what part such overwintering plays in the life history of the fungus.

The problem may be solved by field observations and by experimental methods. The field method presents enormous difficulties, although Brooks (6) and his co-workers gathered much evidence for the persistence of *Phytophthora* in the field during their investigations. However, the study of the development of the fungus under different conditions in the laboratory is also of much value. In this respect the discovery of the occurrence of oospores in pure cultures by Clinton (11) and by Pethybridge and Murphy (26) has been of great importance, since the failure to find resting spores was one of the principles underlying De Bary's opinion. The discovery of an oospore in pure culture on sterilized soil (8) increased the probability of the occurrence of oospores under natural conditions.

The saprophytic life of the fungus is closely related to the problem of hibernation. The author has proved that *P. infestans* is not so strict a parasite as was thought formerly. It was also ascertained that it can live in pure cultures on different types of soil (7). The existence of the fungus in the soil, and its growth there in the absence of living potatoes, strengthens the idea of hibernation independent of the potato tuber.

The great problem concerning overwintering which remains to be solved, however, is the question whether the fungus can endure the low temperatures of winter. No definite results were obtained on this matter in previous work. Following this, the question arises as to the form in which the fungus can withstand the cold. Are oospores necessary, and under what conditions are they formed? The present investigation was undertaken to settle these questions, since more exact methods were now possible in low temperature experiments, owing to the availability of a refrigerator. At the same time, additional information was obtained concerning the saprophytic life of the fungus.

THE SAPROPHYTISM OF PHYTOPHTHORA INFESTANS

About the beginning of the present century, various investigators succeeded in growing the blight fungus in pure culture on different media, thus proving that it is not so strict a parasite as was thought formerly. Most of the investigators considered its saprophytism to be rather narrow. Enumerating the various media used, however, makes it evident that a great variety of substances are able to nourish the fungus. *P. infestans* may require special conditions for its growth, but is not restricted to a few substances for food material.

Good growth was observed on many plant media, viz., potato, oat, maize, lima and white bean, Spanish melon, and vegetable marrow [Matruchot and Molliard (23), Brefeld (5), Clinton (9) (10) (11), Jones, Giddings, and Lutman (13), Pethybridge and Murphy (26)]. These substances were prepared in different ways and used alone, moist, or mixed with agar or gelatin. The potato, Spanish melon, and vegetable marrow were also used in living condition.

Heeke (12) used decoctions of plums, tomatoes, cherries, and potato leaves in moist chambers and in watch glasses for the study of the germination of the conidia. He writes: "By transferring these several times one gets at last a pure culture." From this it is not clear whether Heeke really grew the fungus in pure culture on these media for any length of time. Attempts by the present author to grow the fungus in plum and cherry decoctions of different concentration or neutralized with KOH gave unsatisfactory results. Plum and cherry decoctions, therefore, are not being added to the list of media on which *P. infestans* can thrive.

In addition to those made from vegetable material, synthetic media were used. Matruchot and Molliard (23) state that the fungus grew in a three per cent solution of glucose in water. Jones, Giddings, and Lutman (13) describe good or fair growth on some ten synthetic media of varying composition, while Kossowiesz (14, 15, 16, 17, 18, 19, 20, 21) used about 50 different combinations successfully. Kossowiesz proved that *P. infestans* can utilize uric acid, hippuric acid, glycocol, or guanin as sole sources of carbon and nitrogen; that urea, guanidin, potassium sulphocyanide, nitrite, nitrate, or calcium cyanamide can be the sole sources of nitrogen; and that as the sole carbon compound in the solution, either saccharose, dextrose, or mannite suffices. *P. infestans* therefore does not require special substances as nutriment, but can obtain the necessary carbon and nitrogen in quite different forms.

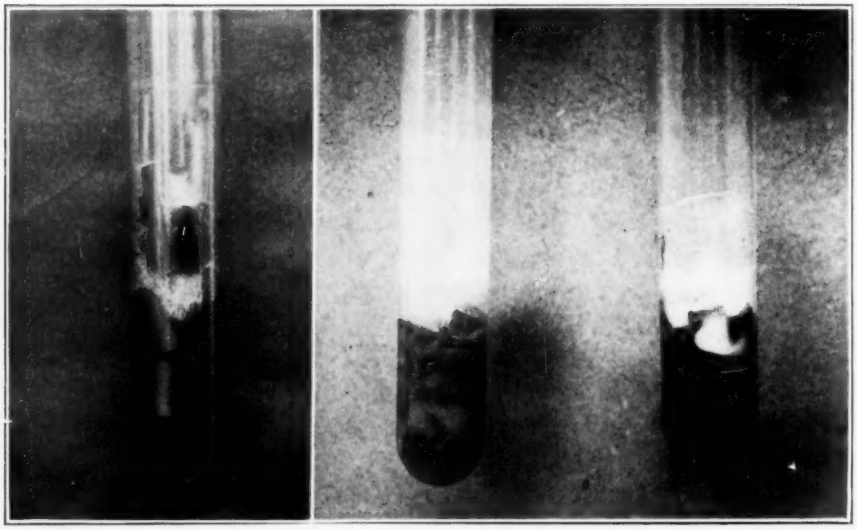
Kossowiesz used liquid media. Most of the earlier investigators were of the opinion that it made no difference whether liquid broth or solidified agar or gelatin were used. Clinton preferred agar to gelatin. Löhnis (22) stated that she obtained better results when one instead of two per cent agar was used. According to the writer's experience, *P. infestans* requires rather moist media. Although agar or gelatin may give good growth, the fungus grows better on a mixture of firm material with liquid. For the experiments described here the standard medium consisted of three or four maize kernels in an excess of water. A thick mat was formed on the surface of the liquid, and pieces of this mat were very handy for transferring.

The growth of *P. infestans* in pure culture on different types of soil (7) also is of importance in relation to its saprophytic life. Bog soil, clay, and leafmold proved to be better media than sand, although on the last named slight growth occurred. Best of all is sterilized manure, which could be added to the list of media in 1923 (8). The fungus has remained alive on bog soil with no other food material for nearly four years, and has been transferred only when the soil in the tube became dry. On manure the fungus has now been living for two and one-half years. The constituents of the soil, as well as those of manure, suffice as nourishment for the fungus.

Since the organic parts of the soil form probably the chief substances on which the mycelium feeds, and since these consist partly of old plant remains, attempts were made to determine to what extent portions of different plants could be used as media for growth. The condition of the plant before sterilization was observed to influence the behavior of the fungus. Parts taken from living plants gave different results from those obtained when dead and discolored parts of the same kind of plant were used. The

plants were cut into pieces, placed in tubes and covered with distilled water. Enough water was used so that after sterilization only the upper portions were above the water level. The fungus was transferred to these tubes from the stock culture on maize kernels.

On the best media, growth was noticeable within a few days after inoculation. On certain others no growth could be recognized at the beginning, although it started later and resulted, in the end, in very good mycelial development. It made no difference whether the tubes were used immediately or some months after sterilization. On certain media only



a b

FIG. 1. a. Culture of *Phytophthora infestans* on wheat straw.
b. Culture of *Phytophthora infestans* on fresh and dead stems of the same variety of potato, a fortnight after inoculation.

slight growth took place at the beginning and practically no increase of mycelium occurred during six months. In some of these cases of very dubious growth, the original piece of mycelium was transferred after four and one-half months to a more suitable medium. Excellent development resulted, thus proving the inhibitory action of the previous medium on the growth of the fungus, and the strong vitality of the organism. There also is a difference in the proportion of tubes in which the fungus developed. On the good media, growth was obtained in all the tubes inoculated, while in other cases growth was observed in only a very small number of tubes.

Table 1 gives a list of the plants used and the results obtained (Figs. 1 and 2).

TABLE 1.—*Growth of Phytophthora infestans on various sterilized vegetable media*

Medium	Character of growth
Oat straw	Very good
Wheat straw	do
Rye straw	do
Rye haulms; fresh	Good in 50% of the tubes
Grass; fresh	Fair
Potato stem; dry, brown	Very good
do fresh, green	Slight after one month, good after two months
Equisetum; dry, brown	Very good
do fresh, green	Very slight
Bean stem; dry, brown	Good
do fresh, green	Slight after one month, fair after two months
Horse bean stem; dry, brown	Good
do fresh, green	Very slight after two months in two tubes out of eight
do pod; dry, black	Good
do pod; fresh, green	Slight after one month, good after two months
Rhubarb flower stalk; dry, brown	Good
do petiole; fresh, green	None
Chrysanthemum leucanthemum; dry, brown	Good
do fresh, green	Very slight after three months in one tube out of six
Beet root; fresh	None
do petiole; dead	do
do do fresh	do
do flower stalk; dry from previous year	Good
do fresh	None
Tomato fruit;	None
do stem; dry	Very good
do fresh	Good after three months in one tube out of seven
Carrot root	Fair
Pteridium aquilinum; dry, brown	Good
Calluna vulgaris; dry, brown	Slight in one tube out of six
Larix needles; dry, brown	None
Mixture of various plants gathered at the side of a ditch; dry, brown	Good

The most striking fact is the far better growth of the fungus on sterilized, old, dried, brown parts of plants than on sterilized fresh plant parts. In some cases the fungus flourished on the sterilized, withered plant re-

mains, while no growth whatever took place on the same kind of plant sterilized in fresh condition. When growth occurred on fresh plant parts, it was always much less than that which occurred when the fungus was grown on dead parts. It is evident that the fungus prefers partially decayed plants, and this leads to the conclusion that, on the whole, *P. infestans* possesses more saprophytic than parasitic tendencies.

The explanation of this fact must be either that the substances in the plants are altered to such an extent through withering that only after this can they be utilized as food by the fungus, or that the fresh plants contain substances which inhibit growth. Since *P. infestans* can grow in solutions of widely varying and rather simple composition, it is not very likely that food material is lacking in any plant decoction. It is highly probable, therefore, that the second supposition holds true.

The inhibiting factor may be either some specific substance or the concentration in which one or more of the substances are present. Jones, Giddings, and Lutman (13), as a result of their experiments with synthetic media, are of the opinion that a high concentration of a solution prevents growth. Löhnis (22) disproved this by cultivating *P. infestans* on bean agar with 60 per cent of sugar. Although high osmotic pressure of itself may have no inhibiting effect, it may be true that certain substances become toxic if present above a certain concentration. The fact that in some cases development occurred in only a very small number of tubes may point in this direction, as also may the slight growth on some media. However, in those cases where good growth started after one or two months the explanation is rather difficult.

In connection with the study of the resistance of the potato to blight, it is important to know what factor really inhibits growth.

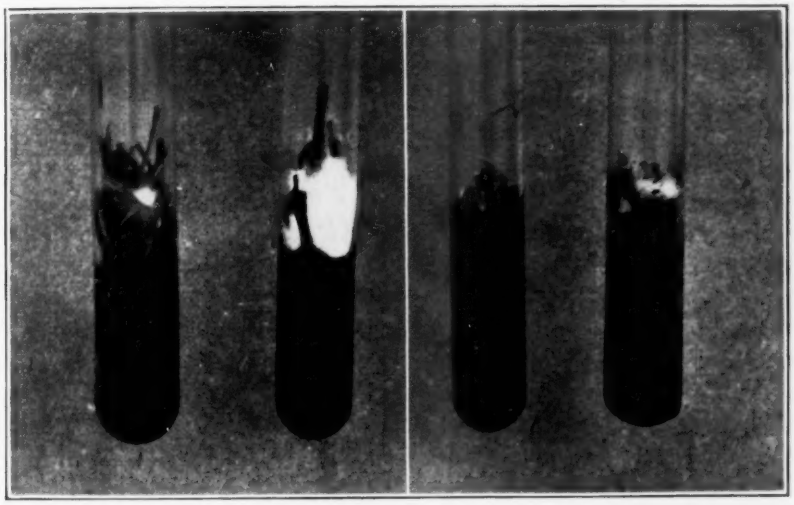
The number of plants which can serve as nutriment is large, as can be seen from table 1, and they do not belong to closely related families. The plants used were, on the whole, only those which happened to be at hand in both conditions, brown and dead, and fresh and green, respectively.

The experiment shows that a saprophytic life of *P. infestans* is quite possible in the field, independent of the potato plant. In all types of soil some of the plant remains mentioned will be found in all stages of decay, especially the straw of cereals and grasses. The quantity of suitable organic constituents may vary according to the type of soil and treatment of the field, but some of them will be present in all soils.

In admitting the possibility of the fungus living in the soil, no difference is assumed in the behavior of the fungus towards soil in a sterilized state and under natural conditions. Competition with other organisms might check growth in unsterilized soil, but whether *P. infestans* would

lose or win in this struggle for existence is unknown. In the author's experiments the fungus was found on the whole to possess strong vitality.

Murphy and McKay (25) object to the idea of the fungus being able to live in the soil for long periods. Murphy (24) had carried on experiments with unsterilized soil, and came to the conclusion that soil contaminated with conidia of *P. infestans* retains its power of infection for forty-four days. According to him, it is the formation of germ tubes and secondary conidia which prolongs the vitality of the spores. He does not accept the idea of a growth of the fungus through feeding on the soil contents. His results, however, can be explained as easily by a saprophytic life of the fungus in the soil as by the prolongation of its existence by the formation of secondary conidia. It may have been due to unfavorable conditions that, in Murphy's experiments, growth stopped after forty-four days. Finely sifted potting



a b

FIG. 2. a. Culture of *Phytophthora infestans* on fresh and dead *Equisetum*, a fortnight after inoculation.
b. Culture of *Phytophthora infestans* on fresh and dead stems of *Chrysanthemum leucanthemum*, a fortnight after inoculation.

loam and silver sand were used. Both probably contained little organic matter, and therefore but little nourishment. In the present author's opinion the results of Murphy's experiments are not in contradiction with the idea of a saprophytic life of *P. infestans* in the soil.

THE OCCURRENCE OF OOSPORES AND RESTING FORMS

The oospores of *P. infestans* are known only from pure cultures. They were observed on oat-juice agar, Clinton (11), ground Quaker oats agar, Pethybridge and Murphy (26), cornmeal agar and oat-extract agar, de Bruyn (8). Abnormal structures called resting spores were described by Jones, Giddings, and Lutman (13) and by Löhnis (22).

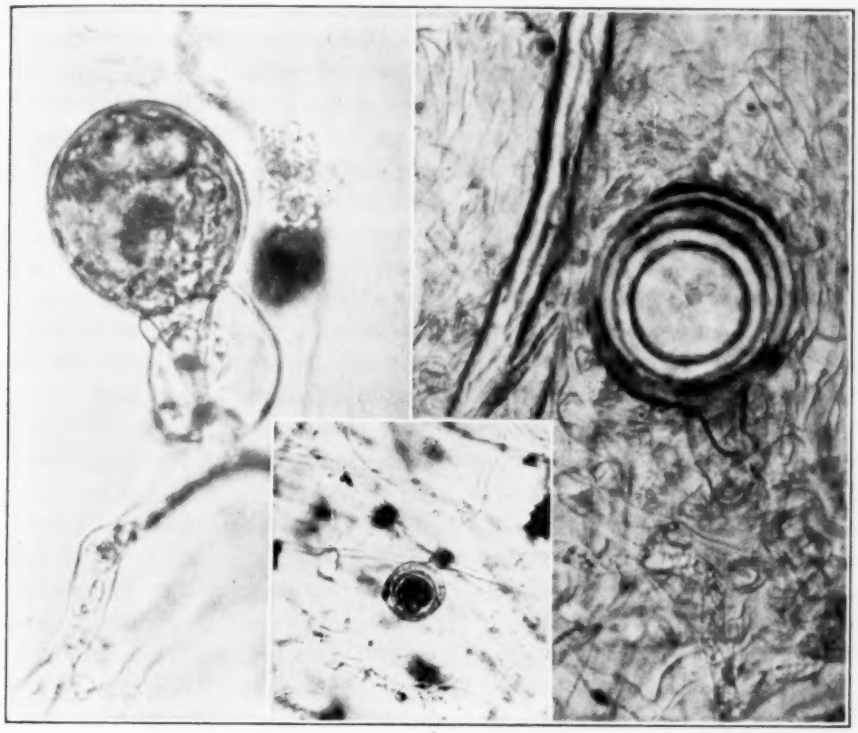
The presence of oospores on the new media used in the present experiments was often very difficult to demonstrate, chiefly when they occurred only in small numbers. The portion of plants used, especially the dead parts, contained all kinds of fungous spores before sterilization, thus making errors very easy. The same held true for the various types of soil. On bog soil (8), however, one oospore developed parthenogenetically and was discovered still attached to the mycelium. (See Fig. 3a.)

Definite oospores with antheridia attached were found in great abundance on the three kinds of cereal straw and on fresh rye haulms. Fig. 3b is an illustration of a young oogonium with an antheridium, from a culture on wheat straw, and shows distinctly that the antheridium is of the amphigynous type. Fig. 3c represents an older stage from a culture on rye straw, the oospore being already formed. The antheridium is also visible.

A tendency to form oogonia was observed on the medium consisting of dead *Equisetum* haulms. In only a few of these oogonia did a thick inner wall develop. Oospores were thus formed parthenogenetically. Very few antheridia were discovered. The bulk of the oogonia did not develop any further. They may be described as dark yellow, round bodies at the ends of hyphae. Their resemblance to true oogonia leads one to regard them as such. The oospore-like bodies mentioned by Rosenbaum (28) as occurring on oat-agar were probably similar. While these oogonia did not develop further, owing to the absence of antheridia, they were abundant in cultures on dead *Equisetum* haulms, and were observed in much smaller numbers on many other media. On dead *Pteridium aquilinum* they were easily found, but a thorough search had to be made on dead and on fresh potato stalks, on dry and on fresh bean stems, on dry *Chrysanthemum leucanthemum*, and on some other media, since only one, two or three were present. It is therefore of no importance to mention all the media on which the oogonia occurred so sparingly, since they may easily have been overlooked in other cases.

The media on which many true oospores were found (viz. the cereals), as well as those on which the fungus showed a tendency to form oogonia (*Equisetum* and *Pteridium aquilinum*), are all media composed of plants with a high percentage of silica. Clinton (11), after finding oospores on oat-juice agar, states that chemical analyses of oats show that they have

higher percentages of ash, fat, and lecithin than the other cereals. It seems that the higher per cent of ash is due largely to silica, and Clinton concludes: "So these constituents of the ash are apparently not the favorable factors." The above mentioned results, however, make it appear very likely that the percentage of silica present in the medium does influence the



b a c

FIG. 3. a. Parthenogenetic oospore from a culture on bog soil × 260.
b. Oogonium and antheridium from a culture on wheat straw × 900.
c. Oospore from a culture on rye straw × 700.

formation of oogonia in *P. infestans*, and probably also affects the production of antheridia and thus the formation of oospores. The quantity of silica, even in plants of the same kind grown under different conditions, will not be uniform. This may afford an explanation of the contradictory results often obtained by various investigators in connection with the formation of oospores on special media. Neither must it be forgotten that all cultures are grown in glass tubes.

In addition to oogonia and true oospores, all kinds of abnormalities are very frequent on the media mentioned and on others. The different types of resting spores described and figured in detail by Jones, Giddings, and Lutman (13) were often found. These resting spores are of very different forms: sometimes they are very difficult to distinguish from oogonia, while in other cases all stages are present, from round, yellow bodies to only slightly swollen or thickened mycelium. The swelling of the mycelium may be intercalary, and in this case the resemblance to a germinated spore is striking. One may as well call this a germinated oogonium as merely a swelling of the mycelium, and it would also be quite justifiable in such a case to speak of a chlamydospore and not of an oogonium. The thickened mycelium may also be strongly branched. Even conidia may have a thickened wall, often, though not always, of a yellow color. Many degrees of development of all these abnormalities exist. One might call them abnormal or not fully developed oogonia, conidia, or mycelium. In many cases the relation to the specific part of the fungus is clear; but, as has been said, in a great number also it is doubtful. It seems best to give them all one collective name, and for this the term "resting forms" is proposed, since this includes the abnormal mycelium as well as the spores. The function of these resting forms will always be the same, the thick wall and the presumed change in the constitution of the protoplasm suggesting adaptation to unfavorable circumstances.

If this name is accepted, the different organs which *P. infestans* may possess are the following: mycelium; conidia; oospores with a thick wall still surrounded by the oogonial wall, being developed either parthenogenetically in the absence of antheridia or sexually when antheridia are present; oogonia with a more or less thick wall; and resting forms, this term to include all those forms which differ from the others and which are characterized by the presence of a thick wall.

These resting forms are found in greater abundance on some media than on others; on certain media also some special form may occur more generally. Next to food, their presence will be dependent on external circumstances and will therefore not always be quite constant. However, there is no need to mention the media and the form on which the fungus occurs on them in more detail.

Since the formation of resting forms is a normal reaction of the fungus to unfavorable conditions, such as unsuitable food or drought, they probably will occur under the same circumstances in nature. It also is very likely that oospores are produced under natural conditions, although the problem of their occurrence in nature has not yet been solved. There is a better chance of finding them on old grass and cereal stubble than on old

potato plants. If oospores are found it remains to be proved that they are really those of *P. infestans*. Not until methods of causing the oospores to germinate have been discovered will it be worth while to make a thorough investigation.

THE BEHAVIOR OF PHYTOPHTHORA INFESTANS IN RELATION TO LOW TEMPERATURES

In 1922 the author (7) published results of experiments concerning the resistance of *P. infestans* to low temperature. Pure cultures of the fungus were exposed to normal winter weather conditions. In 1920–21 the fungus endured a temperature of – 9° C., but during the winter of 1921–22 a temperature of – 12° C. was reached and all the cultures died. The following experiments show that the fungus can withstand a temperature much lower than – 12° C.; and this suggests that the failure in 1921–22 must have been due to mistakes in technique. In the first place, the number of tubes used was too small, because the fungus survives in only a small percentage of cases. Secondly, the cultures were put out-of-doors too soon after transferring, since the fungus probably requires some little time for proper development before it can resist abnormal conditions.

In the following experiments a refrigerator was used; thus it was possible to regulate the temperature and the time during which the fungus was exposed to it. The lowest temperature which could be used was about – 25° C. Since nothing is known about the possible existence of physiological strains of *P. infestans*, the same strain—isolated in September, 1920, from diseased potato plants—was used throughout the experiments to prevent complications in the results.

In the first experiment the influence of the time of exposure, as well as of the treatment of the fungus after freezing, was studied. The whole experiment lasted for ten days, and every two days a certain number of tubes were removed from the refrigerator. Some of the cultures were put at once into an incubator at about 18° C., thus undergoing a sudden change of temperature of about 35° C. The rest were placed for twenty-four hours in a cool cellar at a temperature of – 2° C., next for twenty-four hours in an incubator at + 2° C., then for about ten days in a cellar at a temperature of 6° C., and finally they were transferred to new media and kept in an incubator at about 18° C. The fungus in the first lot of tubes had been transferred after thawing of the medium. The original cultures used in the experiment were also kept in the incubator. It was perceived that in some cases growth started after some weeks in the old tubes, while no development could be observed on the new media. New transfers were made with satisfactory results. In the later experiments, therefore, the cultures

were always kept for a month at the optimum temperature before transfers to new media were made. The fungus was thus able to renew growth and refill the tube. In the first experiment the temperature in the refrigerator was not very constant, varying from −9° C. to −17° C. On each of the first three days, however, −17° C. was reached. All the cultures were at least one and one-half months old, and the majority still older, before they were subjected to freezing. The results are given in table 2.

TABLE 2.—*The effect of keeping cultures of Phytophthora infestans for different lengths of time in different types of soil at a temperature of −9° C. to −17° C., and then thawing them gradually or suddenly*

Cooling period in days	Kind of soil										Totals
	Bog soil		Sand		Leaf mold		Clay		Manure		
	Change of temperature and number of cultures which survived										
	Sudden	Gradual	Sudden	Gradual	Sudden	Gradual	Sudden	Gradual	Sudden	Gradual	
2	2[a]/2	1/2	0/1	0/2	0/1	0/2	0/0	0/1	0/0	0/0	3/11
4	2/2	2/2	0/1	0/2	0/1	1/2	0/0	0/1	0/0	0/0	5/11
6	1/2	0/2	0/1	0/2	1/1	0/2	0/0	0/1	0/0	0/0	2/11
8	1/2	0/2	0/1	0/2	1/1	0/2	0/0	0/0	0/0	1/1	3/11
10	0/2	0/4	0/1	0/4	1/1	0/1	0/0	0/1	0/0	0/0	1/14
Totals	6/10	3/12	0/5	0/12	3/5	1/9		0/4		1/1	

[a] Numerator = number of cultures which survived; denominator = total number of cultures.

The fungus can endure a temperature of −17° C. The length of time during which it is exposed to this temperature is of no great influence since about the same percentage of survival was obtained with two, four, six, or eight days exposure. Only after ten days cooling was there a reduction in the number of cultures in which the fungus remained alive. The difference, however, is so slight that not much stress can be laid on this fact. Growth is somewhat better after a sudden change of temperature than after a gradual change. Richter (27) found, in his experiments with *Aspergillus niger*, that the survival of the fungus after freezing depended on the condi-

tions prevailing after thawing. At optimum temperature immediately after cooling, the fungus started to respire again and to form new mycelium; at a less favorable temperature, *Aspergillus* did not revive. *P. infestans* did not show this sharp difference, although, when placed at the optimum temperature immediately after cooling, it grew somewhat better than when brought to the optimum temperature gradually.

On the whole, the proportion of cultures that survived the low temperatures was rather small. A great difference was found in the behavior of the fungus when grown on various media. This points to the probability that not the mycelium, as in *Aspergillus niger*, but some special form of the fungus may be responsible for its survival, this special form being produced more readily on one medium than on another. This supposition would also explain the rather long time required in some cases for the renewal of growth, and also the irregular and low percentage of survival. Even cultures on the same medium are unequally resistant to cold, a point which is further demonstrated by the next experiment, in which the influence of different temperatures was studied.

TABLE 3.—*Effect of keeping cultures of Phytophthora infestans at different low temperatures for five days*

Medium	Temperature and number of cultures which survived		
	−12° to −15° C.	−17° to −20° C.	−21° to −25° C.
Clay	0[a]/10	0/10	0/10
Sand	0/10	0/10	0/10
Bog soil	2/10	0/10	2/10
Leaf mold	1/5	0/5	0/5
Manure	5/10	7/10	8/10
Maize kernels	0/10	0/10	0/10
Rye straw	1/5	0/5	0/5
Total	9/60	7/60	10/60

[a] Numerator = number of cultures which survived; denominator = total number of cultures.

In this case the temperature was kept as constant as possible at three different levels, viz., $-12°$ to $-15°$, $-17°$ to $-20°$, and $-21°$ to $-25°$ C. Each time the same number of cultures on each medium was used, and they were treated in the same way. The time of exposure to the special temperature was five days. After cooling, the cultures were put at once into the incubator at 18° C., where they were kept for one month to give the fungus opportunity for new development. Transfers were then made to new media. Table 3 gives the results of this experiment.

Not much difference in percentage of survival was obtained at the different low temperatures employed; approximately the same number of cultures resisted $-15°$ C. as $-25°$ C. The great influence of the medium used was again demonstrated. The fungus endures the cold best when grown on manure, next on bogsoil, then on leaf mold. The same result was obtained in the first experiment. It may be added that in another case a culture on clay also resisted $-20°$ C.

Conflicting statements are found in the literature as to the influence of the use of manure on the development of potato blight in the field. In view of the good growth of *P. infestans* on this medium and the strong resistance of the fungus to cold when grown on it, it may be worth while to pay more attention to this point in the future, especially with regard to the influence of manure on the first appearance of blight in the season.

It might be thought that the difference in the resistance of the fungus to cold on the different media was due to a difference of concentration in the decoctions. A correlation between the resistance of fungus mycelium to low temperature and the concentration of the nutrient solution in which they are cultivated was demonstrated by Bartetzko (1), who experimented with four different fungi. In all cases, however, he found a gradual diminution of the percentage of survival corresponding with the lowering of the temperature. The critical temperatures, and the rate at which the percentages decreased, varied with the different concentrations of the solution. He also observed a gradual decrease in percentage of survival in relation to the time of exposure. No such gradual changes were observed in the freezing experiments with *P. infestans*. The relative proportion of cultures that grew after exposure to the three temperatures used, differed only slightly; indeed the lowest gave the highest number of survivals. Neither did the time of exposure exert any observable influence. In Bartetzko's investigations it was the mycelium that resisted the cold in more or less degree according to the concentration of the solution. As has been stated previously, it is probable that the normal mycelium and conidia of *P. infestans* are killed at low temperatures and that it is in some other form that the fungus withstands them. This would explain the behavior of the fungus in the

freezing experiments. It makes no difference in the behavior of these highly resistant organs whether the temperature is – 15° C. or – 25° C. nor whether the exposure to them is long or short.

The oospores are naturally first thought of as being the organs which enable the fungus to remain alive. Manure containing many pieces of straw may hold oospores in abundance. Soils may also contain them in varying number, depending on the presence of silica-containing plant remains. The strange fact remains, however, that the percentage of cultures on rye straw which survived was very small. To obtain further data on this point another experiment was undertaken. This time sixty cultures on different kinds of straw were placed in the refrigerator.

TABLE 4.—*The effect of keeping cultures of Phytophthora infestans dry or moist at a temperature of – 20° C. to – 25° C. for five days*

Medium	Condition of cultures and number which survived	
	Dry	Moist
Rye straw	8[a]/10	0/10
Wheat straw	5/10	0/10
Oat straw	4/10	0/10
Total	17/30	0/30

[a] Numerator = number of cultures which survived; denominator = total number of cultures.

At the same time a new factor was introduced, namely, the influence of moisture and drought. After good development of the fungus, the water was poured out of half the number of tubes. These tubes were kept in a closed vessel containing quick lime, and the lime was renewed from time to time. After one and one-half months all the cultures were subjected to freezing, although those kept in the vessel containing lime were not yet absolutely dry. The cultures were kept in the refrigerator for five days at a temperature of – 20° to – 25° C. After exposure, the dried tubes were filled with sterile water, and then all were placed in the incubator. After a month, transfers were made to new media. The results are shown in table 4.

Very striking is the fact that none of the moist cultures remained alive, while 57 per cent of the dry ones withstood the cold. It is evident that

P. infestans endures low temperatures much better in a dry than in a moist condition. It is a known fact that dry organs are more resistant than moist ones, but it is very remarkable that a fungus like *P. infestans*, which is so dependent on the presence of water for its best development, can endure drought so well. Probably the parts of the fungus which withstand the cold are the same as those which resist drying out. Whether these organs are really only the oospores, is, however, not revealed by the experiment.

TABLE 5.—*The effect of exposing Phytophthora infestans at different stages of development, and growing on different media, to a temperature of −20° C. to −26° C., for five days*

Medium[a]	Form of *Phytophthora infestans* found on the medium in addition to the normal mycelium and conidia	No. of cultures which survived
Potato stem; dead	Very few oogonia, resting forms	1[b]/2
Potato stem; fresh	Very few oogonia, resting forms	1/2
Equisetum; dead	A few oospores; many oogonia, resting forms	0/2
Bean stem; dead	Very few oogonia, resting forms	1/2
Horse bean stem; dead	Resting forms, chiefly many yellow conidia in clusters	1/2
Horse bean pod; dead	None	0/2
Horse bean pod; fresh	None	0/2
Rhubarb flower stalk; dead	Resting forms	0/2
Chrys. leucanthemum; dead	Very few oogonia, resting forms	1/1
Beet flower stalk; dead	Very few oogonia, resting forms	0/1
Carrot root	Very few resting forms	0/1
Mixture of dead plants	Not examined	0/1
Pteridium aquilinum; dead	One oospore, moderate number of oogonia, resting forms	1/4

[a] All media were desiccated except *Pteridium aquilinum.*

[b] Numerator = number of cultures which survived; denominator = total number of cultures.

The fact that none of the moist cultures remained alive was contrary to what was expected, since oospores must have been present in the thirty cultures on straw in considerable numbers. The next experiment gave similar results.

Cultures on different plant media were subjected to a low temperature. They were all desiccated over quick lime, this time for a period of three months. Consequently the media were absolutely dry before cooling. *Pteridium aquilinum*, only, was added afterwards, and thus was frozen in moist condition. The time of exposure was again five days, the temperature varied from $-20°$ C. to $-26°$ C., the lowest used in the experiments. The tubes were treated subsequently as in the preceding experiments. Results are given in table 5.

In table 5 is added the form of the fungus found in old but rather moist cultures on each specific medium, proving that no special correlation exists between the occurrence of oospores and the survival of the fungus. On the media on which a single oogonium or sometimes not even this was formed, the fungus endured the cold; while on *Equisetum*, the only medium in which distinct oospores develop, both the cultures died. Although it cannot be denied that the presence of oospores may have some influence on the resistance of the fungus to low temperatures, yet some other part of the fungus may be highly resistant as well. The resting forms must be considered. With their thick walls, they are equally as well suited as the oospores to protect the fungus from cold, and the constitution of their protoplasm may have changed to such an extent, as to enable it to better withstand the result of freezing. The gradual drying out of the medium may have favored the development of the resting forms. This experiment proved again the great resistance of *P. infestans* to drought.

It is difficult to decide which of the two forms, the oospores or resting forms, is the more important with regard to resistance to unfavorable conditions, or whether both really fulfill the same function. For the present, it is perhaps best to accept the latter supposition.

The results of all the freezing experiments show that the normal mycelium and conidia of *P. infestans* are not able to resist cold, and, therefore, that special organs are required. These may as well be the oospores as the resting forms. In a dry condition they can withstand low temperatures far better than when moist. These forms can endure a temperature as low as $-26°$ C.

Although special conditions are required for resistance of the fungus to cold, it is not impossible that such conditions occur in nature. On suitable plant remains, oospores may be produced. In autumn weather the unfavorable conditions may exist which stimulate the formation of resting

forms. The great resistance shown in the desiccated condition suggests that a dry period preceding frost may keep the fungus alive better than sudden cold after damp weather.

SUMMARY

1. From the experiments described in the foregoing pages, it is evident that *P. infestans* can live saprophytically. It grows best on old, partially decayed plants. In nearly all soils, therefore, organic parts will be present on which the fungus can feed. In addition to the presence of suitable food, temperature and moisture must be taken into account. Moisture is necessary for the good development of the fungus. It is an important factor in producing the best development and also in renewing growth in the spring. In consequence, it is important in connection with the first foliage infection. The fungus can, however, withstand drought as well as cold. The oospores and the resting forms are the resistant organs; both are probably produced in nature. They are able to endure a temperature of $-20°$ C. to $-26°$ C. for five days, and more easily in a dry than in a moist condition.

2. Theoretically, there is no objection whatever to the hibernation of *P. infestans* in the field. It can remain alive outside the potato plant, and endure cold and drought. Its growth is on the whole rather slow, and abundant only in the presence of moisture. However, the fungus is not easily killed. If *P. infestans* finds the right conditions for overwintering on only one thousandth part of a field, this will be enough to produce a blight epidemic in the following year. Under favorable circumstances in the spring, the fungus will renew its growth.

3. If the tuber is the only source from which infection arises, as DeBary held, a careful inspection of seed potatoes may prevent an epidemic of potato blight, especially in a year following a dry summer. The occurrence of overwintering in the field, however, takes away much of the value of this method of controlling the disease, and provides no other in its place, for with present methods it is impossible to kill the fungus in the soil.

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MOSAIC DISEASES ON DIFFERENTIAL HOSTS[1]

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WITH PLATES X TO XIV

In connection with studies on the nature of the causal agency of virus diseases of plants, it became of some interest and importance to know more regarding the specificity of the viruses. In the case of the virus diseases of the potato, it seems evident that a number of quite different viruses are concerned. In the cases of mosaics of other plants, however, there has been a tendency in recent researches either to reduce the number on the basis of results from cross-inoculation studies or to give this phase of the problem minor consideration. In the absence of a more satisfactory method, the classification of viruses must be based largely upon the susceptibility of different hosts, the type of symptom produced on these hosts together with the behavior of the viruses toward various other conditions or circumstances which are applicable as tests. The differentiations must naturally be made under such environment, both with respect to external conditions and the condition of the host itself, and in such numbers that infection may be expected to occur. In some respects these requirements are so exacting as to render their usefulness in classification questionable, especially when employed by different individuals under different working conditions. On the other hand, they are not to be considered more unreliable in this respect than are the methods in use for the differentiation of many bacteria and fungi.

The results presented in this paper deal only with certain mosaics of which tobacco is one of the hosts. In another paper it has already been shown that tobacco and other plants are subject to two, and possibly three, different viruses secured from apparently healthy potatoes. These will not, therefore, be treated in this paper, which will be confined to experiments with the well known ordinary tobacco mosaic and four additional ones hitherto undescribed on tobacco.

SOURCES OF VIRUSES USED

The sources of the viruses with which this paper is concerned are as follows:

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture.

A. Ordinary tobacco mosaic: Originally from tobacco fields on Station grounds at Madison. This form occurs commonly in tobacco fields and there can be little question about its type.

B. Cucumber mosaic: From a mosaic cucumber plant furnished by Dr. S. P. Doolittle, Madison (5).

C. Petunia mosaic: From *Petunia violacea* (double flowered variety propagated by cuttings) grown in Horticultural greenhouses at Madison.

D. Speckled tobacco mosaic: From a tobacco leaf with leaf-spot symptoms sent by Mr. L. R. Tehon of the Illinois State Natural History Survey, Urbana, Ill.

E. Mild tobacco mosaic: From tobacco plants sent by Dr. B. W. Tisdale from Quincy, Florida. These plants were said to be affected with leaf-spots of unknown origin.

The ordinary tobacco mosaic has been used in these experiments primarily for comparative purposes. In this connection, however, some interesting points in its behavior have been observed. The designation of the other types of mosaic on tobacco is regarded as tentative and unsatisfactory. The problem of a system of nomenclature for viruses is a difficult one. We are inclined to believe that designation by letters may eventually be as satisfactory as and less confusing than any name based on host relationship or symptoms. The terminology used by geneticists and by workers with vitamins to designate invisible but definite entities might profitably be followed with virus diseases in plants.

The reader should be reminded, therefore, that two different mosaic diseases of the cucumber have already been reported (7) and that tobacco mosaic has been reported as occurring on petunia (2). The term "speckled" tobacco mosaic is only partly descriptive of the disease, but a more serious objection to the use of a descriptive name for this disease on tobacco is that the symptoms on tobacco are often almost identical with those of petunia mosaic, although differing markedly on other hosts.

The term "mild" tobacco mosaic is fairly descriptive since the type of symptom which occurs on young tobacco plants in the greenhouse is distinctly mild as compared with the other types described in this paper, although it may be confused at times with petunia mosaic.

EXPERIMENTAL RESULTS

It is not deemed necessary to describe in detail the methods used in the experimental work. It is believed that the necessary precautions have been observed. It should be stated, however, that we have relied almost entirely upon needle punctures for inoculations. The plants have been

grown in fertile soil in a warm greenhouse (27–32° C.) and for the most part inoculated when very young and in a vigorously growing state.

For the purposes of this paper, the results are adequately summarized in table 1. In some cases it is recognized that the number of inoculations performed is particularly low as far as negative evidence is concerned. It should be stated, however, that comparative inoculations on which final conclusions are drawn were made simultaneously, thereby reducing the possibility of error. In most cases where the symptoms on a host were of a questionable type, comparative inoculations were made from such plants to other differential hosts, so that, in cases where only one positive infection was secured, its type was verified by evidence that accidental infection with other mosaics had not occurred.

In addition to the results presented in table 1 on differential hosts, considerable evidence of other sorts has been obtained to verify these results. This evidence relates particularly to the length of the incubation period, the "infective power" of the virus (as indicated by percentages of infection secured), and the longevity of the virus in a liquid and in a desiccated condition outside of the living host.

The incubation period on tobacco is, for instance, shortest for ordinary tobacco mosaic, somewhat longer for petunia mosaic and speckled tobacco mosaic, and longest for cucumber mosaic and mild tobacco mosaic. The percentage of infection secured with ordinary tobacco mosaic and speckled tobacco mosaic is on the average much higher than that secured with cucumber mosaic, the others being intermediate in this respect. Ordinary tobacco mosaic virus will retain its infectiousness for years in tobacco extract or in dried leaves, whereas cucumber mosaic is limited usually to less than two days in liquid tobacco extract and to two or three weeks in slowly drying leaves. Petunia mosaic is similar to cucumber mosaic in this respect, whereas speckled tobacco mosaic is considerably more resistant to adverse conditions.

The symptoms of the five mosaics on tobacco are on the whole quite distinct on young plants, except in the case of petunia mosaic and speckled tobacco mosaic (Plate X, A), the symptoms of which sometimes appear to be identical. This similarity also seems to hold on certain other hosts. As the plants grow older the symptoms are often less easily differentiated except in the case of mild mosaic, which rarely produces malformation on young tobacco, although on certain other hosts, such as *Physalis*, this virus is comparatively virulent.

Apparently the viruses which are the most difficult to differentiate are petunia mosaic and speckled tobacco mosaic on the one hand, and petunia mosaic and cucumber mosaic on the other. The former two can be dif-

TABLE 1.—*Summarized results of inoculation*[a] *of various mosaic diseases on differential hosts, with most distinctive symptoms*[b] *manifested*

Mosaic	Hosts										
	Tobacco *N. tabacum*	*N. glauca*	*N. glutinosa*	*N. rustica*	Pepper *C. annum*	Tomato *L. esculentum*	*Physalis pubescens*	Petunia *P. violacea*	Nightshade *S. nigrum*	Eggplant *S. melongena*	Pokeweed *P. decandra*
Ordinary tobacco	85 / 84 m, mf, s	10 / 10 s	15 / ± 8 n	10 / 10 n, m, mf, s	8 / 8 m	15 / 15 m, s	5 / 5 m, s	5 / 5 m	5 / 5 m	15 / 10 n	10 / 0
Cucumber	105 / 57 c, m, mf	5 / 2[c] mf	10 / 3[c] m, mf, s	10 / 5 c, mf	8 / 6 m	13 / 7 mf	10 / 4 m, mf	5 / 5 mf, s, n	10 / 1[c] m		20 / 16 m, s, n
"Speckled" tobacco	100 / 91 m, s	10 / 10 m	20 / 0	10 / 9 mf, s, n	10 / 0	10 / 6 m	15 / 2?	10 / 10 mf, s, n	5 / 5 m	16 / 0	10 / 0
Petunia	65 / 40 m, s	10 / 10 m	20 / 6 m, mf	10 / 9 mf, s, n	10 / 0	10 / 4 m	20 / 4 m, mf	15 / 7 m	5 / 5 m	16 / 0	10 / 2 c, n, s
"Mild" tobacco	45 / 24 m	5 / 0	15 / 7 c, s, n	10 / 4 m, mf	5 / 1[c] m	5 / 4 m	5 / 5 c, s	5 / 0	5 / 1[c] m	5 / 0	10 / 0

[a] Upper figure is number of plants inoculated; lower figure number of plants infected.

[b] C = chlorosis; M = mottling; N = necrosis; Mf = malformation; S = stunting.

[c] Type verified by inoculation.

ferentiated on *N. glutinosa*, petunia (Plate XII), pokeweed and probably on *Physalis*, as well as by their comparative longevity outside of the host. The two latter are best differentiated on *N. rustica*, tomato, or petunia.

Other solanaceous hosts have been tried, but on the whole have not been used sufficiently to warrant including them in table 1. It should be stated, however, that tobacco mosaic was found to produce symptoms on *Solanum rostratum* (new host), while it failed to give mottling on *Datura stramonium*, although it produced striking lesions on the stems of young plants when applied to them with slight wounding. Similar lesions were produced on various other plants, including potato and pepper. In the case of young plants of *N. glutinosa* and *N. rustica*, this virus is frequently capable of killing the plants as a result of stem lesions, even though, peculiarly enough, in the case of *N. glutinosa*, the virus is not apparently present in the foliage. Tobacco mosaic produced distinct stunting but little, if any, mottling on *N. glauca*. It affects egg plant only with difficulty, mottling rarely developing, although stunting and stem or leaf necrosis may be very striking on young plants.

Cucumber mosaic usually produces larger chlorotic areas on tobacco than tobacco mosaic, and these areas are frequently more distinct near the tips of the young leaves. The symptoms of cucumber mosaic on *N. glutinosa* are especially striking with respect to mottling and malformation. On tomato, cucumber mosaic frequently produces the characteristic foliage malformation sometimes referred to as the "maiden-hair fern" type (Plate X, C). Ordinary tobacco mosaic has not yet produced this type of symptom on tomato in our experiments. As compared with the other species studied, petunia and pokeweed appear to be especially susceptible to cucumber mosaic. Speckled tobacco mosaic also is virulent on petunia. In one series of inoculations, all of the five plants were killed. Petunia mosaic, on the other hand, seemed to be only mildly effective on the petunia plants used. It should be stated, however, that the variety used was heterozygous in nature and not the same as that from which the mosaic originally came.

The behavior of the speckled and petunia mosaics on *N. glauca* is interesting in that the symptoms of mottling are very faint and sometimes accompanied by a scattered purplish tint as compared with the controls.

The mild mosaic on tobacco is so readily masked that symptoms often apparently disappear and reappear intermittently over short periods of time, depending apparently on minor environmental variations. On *N. rustica*, however, decided malformation may occur, and young plants of *N. glutinosa* may be killed or stunted without occurrence of mottling. While the data on *Physalis* is limited, the symptoms were so marked that there can be little question but that it should be regarded as a good differential host for mild mosaic (Plate XI).

DISCUSSION

The results presented are believed to justify the conclusion that we have been dealing in these experiments with five separate viruses. It has been shown in another paper also that two and possibly three additional ones have been secured from apparently healthy potatoes. There are in all probability several more viruses which will eventually be found to attack tobacco and other solanaceous plants, since we have not yet made a preliminary survey of this field of investigation, utilizing only those specimens which were conveniently at hand during the winter months or which were sent to us for determination by other workers.[2] It might be argued that a virus is not a definite entity but is so modified by its hosts or other conditions that cross-inoculation experiments of this sort are of little actual significance. Our experience with these viruses, carrying them through several generations of the same and different hosts, is quite contrary to this suggestion, although it is admitted that this important possibility merits more special consideration than we have been able to devote to it.

It is believed that the results presented here suggest a reasonable explanation for some of the contradictory results secured by some workers with tobacco mosaic. This may best be illustrated by the experience of certain investigators with the mosaic disease of *N. glutinosa*. Allard (3) found this species (the *N. viscosum* of Allard's paper) immune from tobacco masaic, but reported it to be affected with a mosaic distinct from tobacco mosaic. Walker (9) and later Elmer (6) question Allard's original conclusion. Our experiments on this subject indicate that Allard was essentially correct in his interpretation. Tobacco mosaic apparently is not capable of acting as a systemic invader of *N. glutinosa*, although it may cause very marked necrosis of young stems; the virus is apparently not present in the leaves (Plate XIII, A). Many other plants are affected by ordinary tobacco mosaic in a similar manner, but such lesions cannot be said to represent true susceptibility to mosaic.

It has been shown in this paper that cucumber mosaic and petunia mosaic produce typical symptoms of mosaic mottling and malformation on *N. glutinosa* (Plate XIII, B), and it is not unlikely that one of these—most likely the former—is Allard's mosaic on *N. glutinosa*, as well as the one used by Walker and by Elmer. We have, in fact, considerable evidence in that direction, which is not deemed sufficiently important to be presented in detail here.

The necrotic lesions produced on stems of various plants by tobacco mosaic is especially interesting and merits special study. This occurs ap-

[2] Since this paper was written, at least three additional viruses have been found to occur on tobacco.

parently on species which are true hosts as well as on others which are not true hosts. These lesions have been observed principally upon potato, *Datura*, pepper, *N. glutinosa* and *N. rustica*.

N. glauca has been regarded as a typical "carrier" of tobacco mosaic, *i.e.*, carrying the virus without itself showing symptoms. *N. glauca*, however, may become very markedly stunted by tobacco mosaic, while all other symptoms usually are lacking (Plate XIV). *N. glauca* is also susceptible to cucumber mosaic, which produces distinct mottling and malformation, as well as to other virus diseases producing more marked leaf symptoms than does tobacco mosaic. Our experience with this and other species indicates that our understanding of true virus "carriers" (1, 8), as distinct from masking and other conditions, is so limited and uncertain as not to warrant the use of the term "carriers" in a sense distinct from plants with masked symptoms.

The recent work of Elmer (6) in particular tends towards the conclusion that little specificity is shown by mosaics from a wide range of hosts. As far as known, however, it has not yet been definitely shown that ordinary tobacco mosaic has been transmitted to hosts outside of the solanaceous family. On the other hand, cucumber mosaic is apparently capable of infecting plants in widely different families. The results presented in this paper show that tobacco, like the potato, may be host to a number of different virus diseases. These observations merit careful consideration in any theory developed as to the nature of the causal agency of virus diseases in plants.

SUMMARY

1. Tobacco, tomato and other solanaceous plants, aside from the potato, have been generally believed to be affected with only one mosaic disease, namely, tobacco mosaic. This paper presents evidence that at least 5 distinct mosaics may occur on various species of this family in addition to other virus diseases not described in this paper.

2. The four additional mosaics are: cucumber mosaic, petunia mosaic, "speckled" tobacco mosaic and "mild" tobacco mosaic.

3. These mosaic diseases are shown to be different from tobacco mosaic and from each other primarily through their behavior on differential hosts.

4. It is believed that these results demonstrate that it is important in future researches with mosaic to differentiate and describe more specifically the type of mosaic used in experimental work.

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EXPLANATION OF PLATES

PLATE X

A. "Speckled" tobacco mosaic on tobacco. This type of mottling is somewhat characteristic for this mosaic as compared with ordinary tobacco mosaic.

B. Tobacco mosaic. A common type of mottling. The variation in symptoms is so large, however, that "speckled" and ordinary tobacco mosaic cannot always be distinguished on tobacco.

C. Upper and lower leaves from tomato plant infected with cucumber mosaic. Note type of malformation in upper leaf and chlorosis and stunting in lower leaf. This symptom has been fairly distinctive under the conditions of the experiments described in this paper.

D. Upper and lower leaves of healthy tomato plant for comparison with C.

PLATE XI

A. Healthy controls of *Physalis* and *N. glutinosa*.

B. *Physalis* and *N. glutinosa* plants infected with the "mild" type of tobacco mosaic. Marked stunting and chlorosis occurs on *Physalis*, but only moderate stunting and chlorosis on *N. glutinosa*.

C. *Physalis* and *N. glutinosa* plants infected with petunia mosaic. Note stunting, malformation and mottling on both hosts in comparison with controls (A).

PLATE XII

A comparison of petunia mosaic and "speckled" mosaic.

A. *N. rustica* inoculated with petunia mosaic.

B. *N. rustica* inoculated with "speckled" mosaic.

C. Petunia inoculated with petunia mosaic.

D. Petunia inoculated with speckled mosaic.

E. *N. glutinosa* inoculated with petunia mosaic.

F. *N. glutinosa* inoculated with "speckled" mosaic.

Note that while the symptoms are apparently identical on *N. rustica*, they differ markedly on the other plants.

PLATE XIII

A. *N. glutinosa* inoculated with ordinary tobacco mosaic.

B. *N. glutinosa* inoculated with cucumber mosaic.

C. Control, not inoculated.

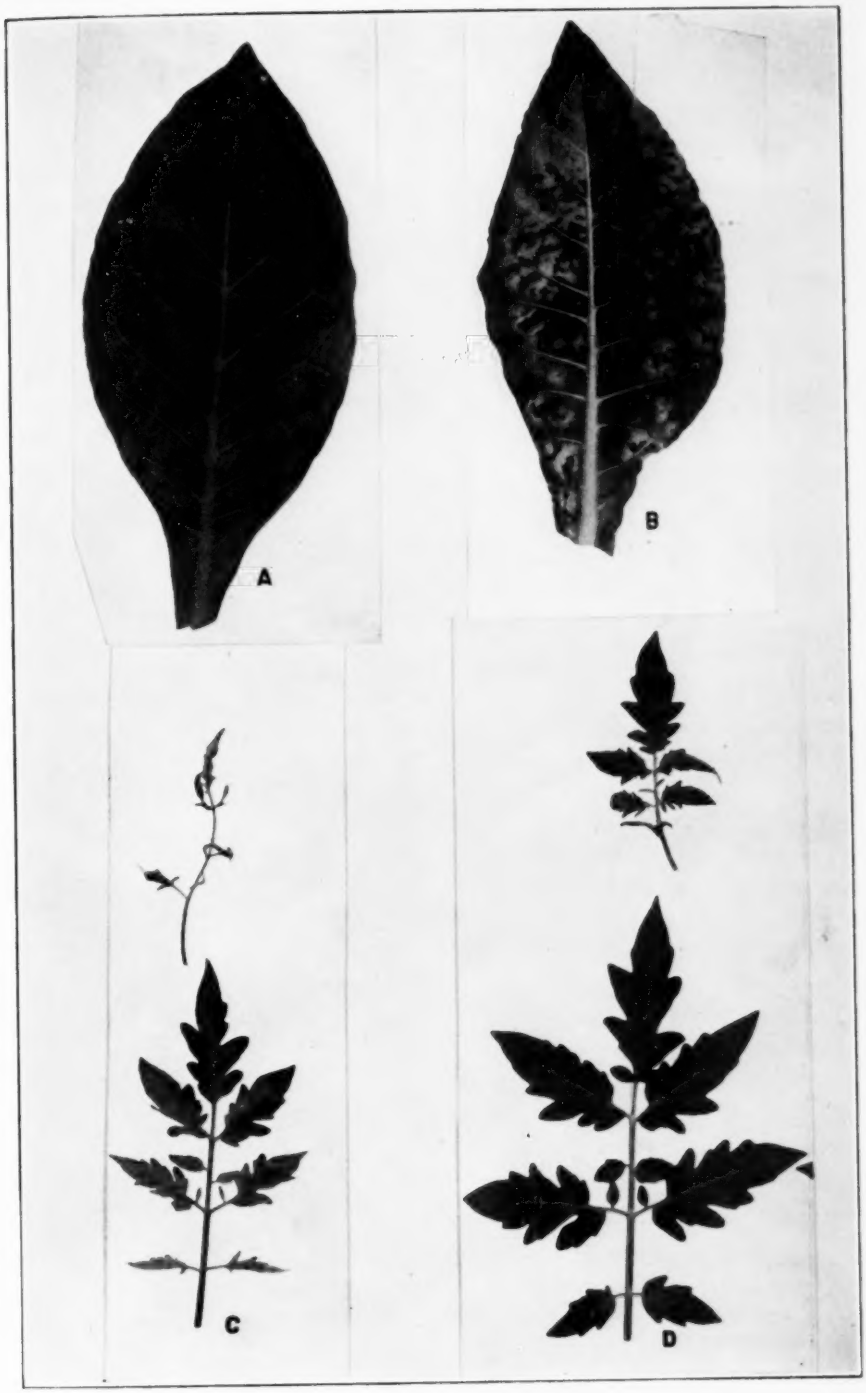
D. *N. glutinosa* inoculated with "ring-spot" virus secured from apparently healthy potatoes.

Ordinary tobacco mosaic frequently kills young *N. glutinosa* plants due to necrotic action on stems, yet the virus is apparently not systemic in the leaves. The type of virus disease shown in D illustrates the distinctiveness of still other types besides those described in this paper.

PLATE XIV

A. *N. glauca*, uninoculated control.

B. *N. glauca* inoculated with ordinary tobacco mosaic showing faint chlorosis of leaves but more particularly marked stunting of growth.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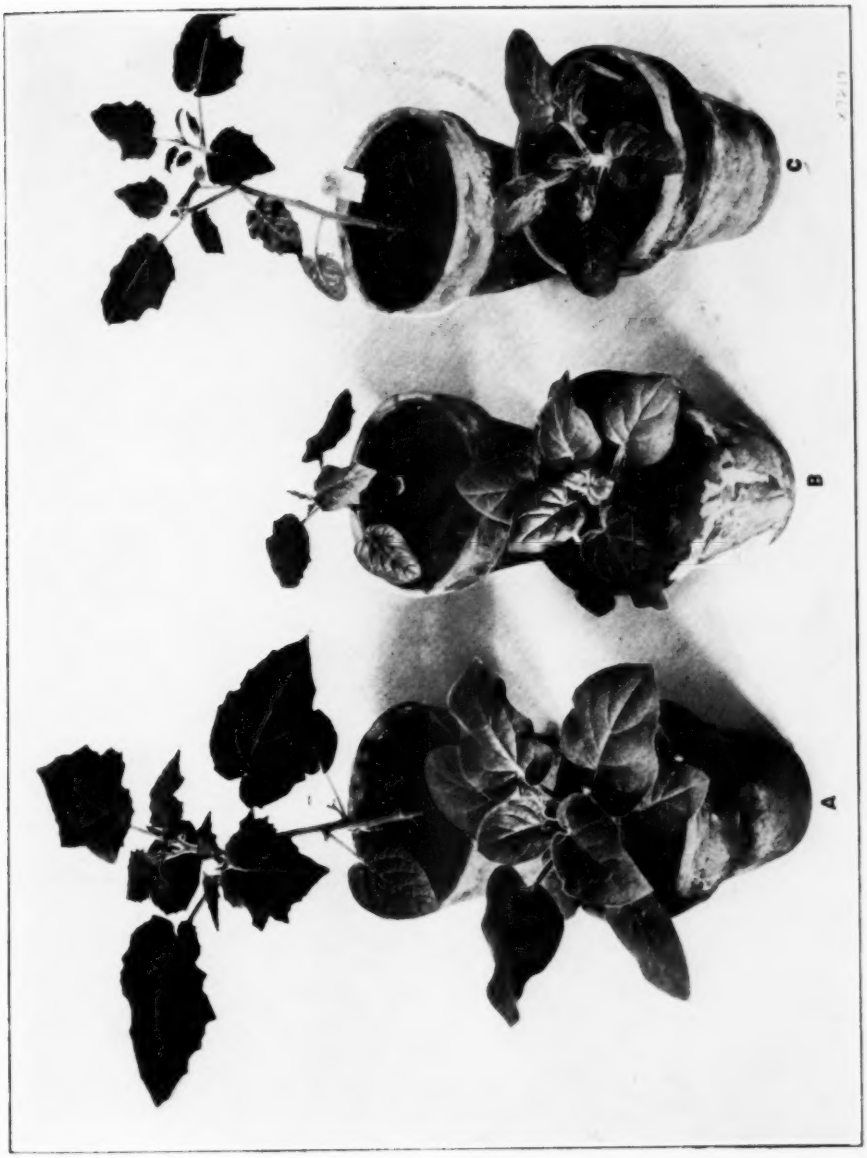
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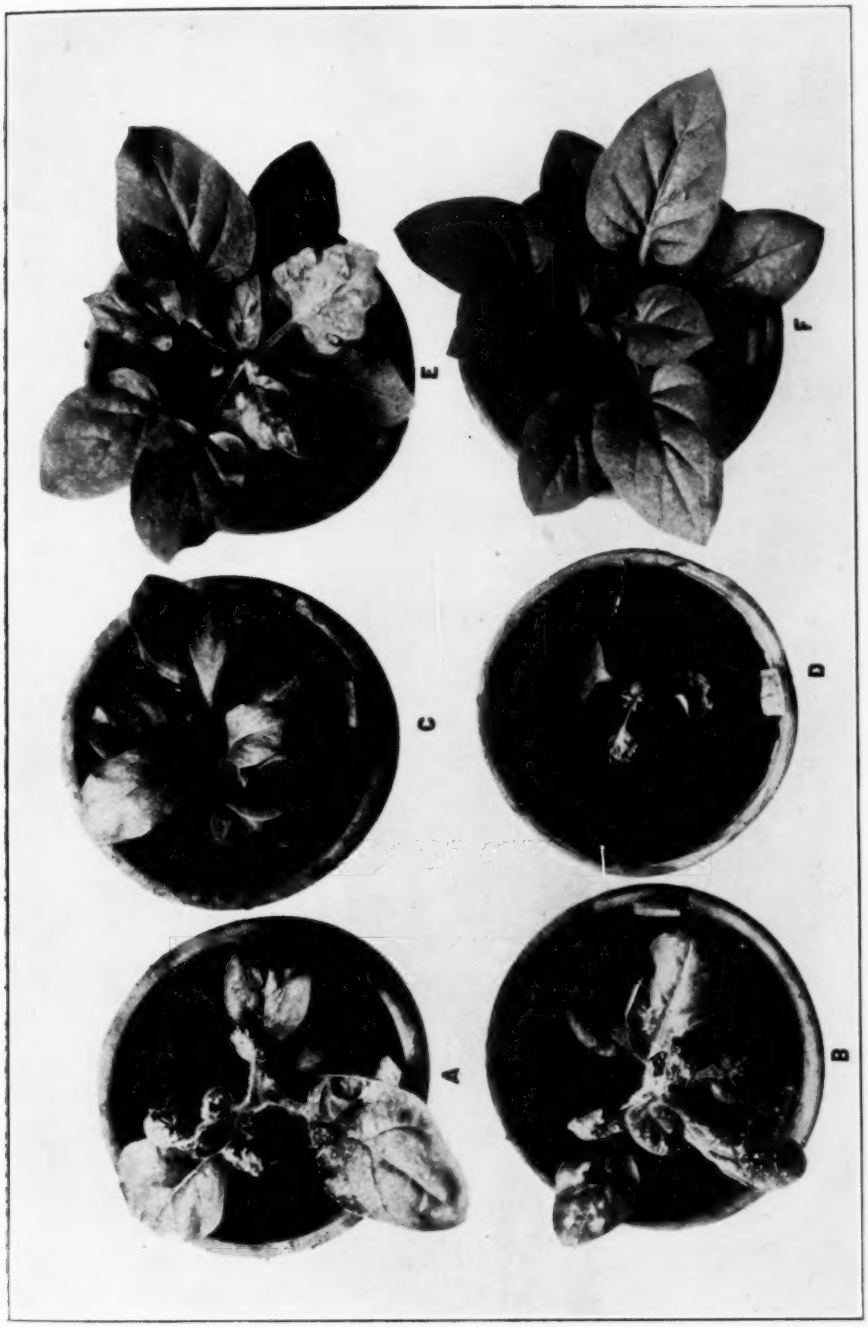
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